REMARKS/ARGUMENTS

Reconsideration of this application and entry of this Amendment are requested. Claims 116-613 will be pending subsequent to entry of this Amendment.

Amendments

Claim 71 (now 116) has been amended by essentially incorporating the limitations of claims 72 and 73, which have been deleted. The term "consisting of" has been replaced by the original term "comprising", in order to make the claim congruent with claims 77 (now 120) and 78 (now 121) . The terms "possible" and "relative" have been deleted. Other minor amendments have been made to correct typing errors, for example "N is the number..." has been corrected to "n is the number...".

Claim 75 (now 118) has been amended by deleting the compounds disclosed in the cited references.

Claims 76 (now 119), 99 (now 131), 102 (now 134), 109 (now 140) and 112 (now 143) have been corrected with the proper name of the claimed camptothecin. Originally, a typing error recited 7-butoxyiminomethylcamptothecin. However, Example 9 in the specification shows the preparation of this camptothecin starting from 7-formylcamptothecin and O-**t-butyl**hydroxylamine hydrochloride. This correction is also confirmed by the attached EP 1 044 977, application n. 99830124.6, cited on page 28, lines 1-2 of the specification. Example 2 of the EP patent is exactly the same of Example 9 of the application. On page 12 of the EP patent the compound of Example 9 of the application is clearly identified with the same code (CPT184).

Analogously, claim 86 (now 122) has been amended in view of claims 87 and 88, which have been deleted. The term "consisting of" has been replaced by the original term "comprising", in order to make the claim congruent with claims 87 and 88. The terms "possible" and "relative" have been deleted.

Claim 90 (now 124) has been amended by deleting the compounds disclosed in the cited references.

Analogously, claim 94 (now 128) has been amended in view of claims 95 and 96, which have been deleted. The term "consisting of" has been replaced by the original term "comprising", in order to make the claim congruent with claims 100 (now 132) and 101 (now 133). The terms "possible" and "relative" have been deleted.

Claim 98 (now 130) has been amended by deleting the compounds disclosed in the cited references.

New claims 116-124 (now 147-155) have been added. Claim 116 (now 147) is directed to a specific embodiment of the present invention, namely a method of transporting an antitumor drug to the **lungs** of a subject in need of antitumor treatment, wherein said drug is selected from the group consisting of taxol or a camptothecin derivative disclosed in the specification, and the liposome is limited as per amended claim 71 (now 116). Claims 117-124 (now 148-155) essentially correspond to claims 97-104 (now 129-136).

Support for these amendments are found in the original specification, *see* in particular pages 55-63.

New claims 125-132 (now 156-163) have been added. Claim 125 (now 156) is directed to a specific embodiment of the present invention, namely a method of **intracellular delivery of an antitumor drug into tumor cells to the lungs** of a subject in need of antitumor treatment, wherein said drug is selected from the group consisting of taxol or a camptothecin derivative disclosed in the specification, and the liposome is limited as per amended claim 71 (now 116). Claims 126-131 (now 157-162) essentially correspond to claims 97-104 (now 129-136).

Support for these amendments are found in the original specification, *see* in particular pages 55-63.

No new matter has been added.

Claims116-163 are now under consideration.

Claim Rejections – 35 USC § 112 – first paragraph

By the above amendments, the rejection has been made moot. No negative limitations are recited.

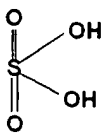
Claim Rejections – 35 USC § 112 – second paragraph

By the above amendments, the rejection has been made moot. The term "comprising" makes claims 76 (now 119), 86 (now 122) and 94 (now 128) are now consistent with all dependent claims.

The deleted terms "possible" and "relative" remove any concern on clarity.

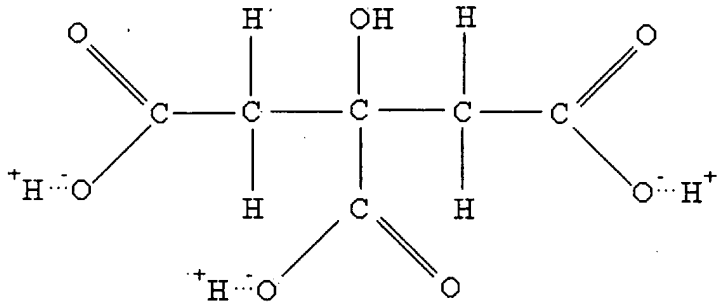
The terms "sulphate" and "acid sulphate", "citrate" and "acid citrate" are clear to the person of ordinary sill in the art.

Sulphuric acid is a diprotic acid: H_2SO_4,



wherein two protonic hydrogen atoms are each bound to an oxygen. When dissociation occurs, the first proton is released, thus the molecule is HSO_4^-. This form is called "acid sulphate" or "disulfate". When dissociation further progresses, the second proton is released, thus the molecule is SO_4^{2-}. This form is called "sulphate".

Citric acid is a triprotic acid:



wherein three protonic hydrogen atoms are each bound to an oxygen. When dissociation occurs, the first proton is released and this form is called "acid citrate".

In view of this clarification, the rejection is not moot.

The term 7-butoxyiminomethylcamptothecin has been corrected. Applicants thank the Examiner for the suggestion.

Claims 75 (now 118), 90 (now 124) and 98 (now 130) have been corrected by deleting the formerly excluded compounds and also in view of the current amendments of independent claims.

Claims 71-78 (now 116-121) and 86-105 (now 122-137) – 35 USC § 103(a)

Wang et al in combination with Allen, Burke, in further combination with Stracher et al

Examiner's rejection will be further discussed by referring also to the previously presented remarks, which will not be repeated here, but still relied on.

In the pending Office Action, the Examiner did not discuss all the arguments presented by the Applicants, therefore the maintenance of the rejection is not completely and accurately legally based. *See* 37 CFR §1.104(b)[1] completeness of examiner's Action and MPEP §707.07(f)[2].

In their last reply, Applicants pointed out the importance of alkyl/acyl chain coupling for delivery efficiency as taught in Wang et al. (*see* page 2211, right column last but one paragraph). As now claimed, the liposomes are not following the rank taught by Wang, however, they show brilliant intracellular delivery into tumor cells of taxol and camptothecin, as demonstrated by increased anticancer activity (*see* specification, page 57, where increased *in vivo* activity on tumor metastases is shown; pages 58-61, where efficiency is shown as comparable to organic solvent DMSO, which solvent of course cannot be used in treating patients; and pages 62-63, where lung tropism is shown.)

In order to find an efficient liposome for intracellular delivery in tumor cells, the person of ordinary skill in the art would not have selected the claimed liposomes in view of Wang et al. teaching on the selection of the alkyl/acyl chain.

In fact, the now claimed subject-matter does not overlap with the compounds of Wang et al and demonstrates that, contrary to the compounds of Wang et al, the efficiency of delivery does not follow the rank alkyl/acyl chain C14 > C12 > C18/C18(9) > C16 > C18 for delivery efficiency.

The Examiner did not specifically comment on Applicants' arguments on Allen et al. For completion of the record and as said in our replies of June 22, 2007 and of June 5, 2008, Allen et al. disclose a therapeutic liposome composition comprising:

1. a pre-formed liposome entrapping a therapeutic agent;

2. a conjugate composed of:

(a) lipid with a polar head and a hydrophobic tail,

(b) hydrophilic polymer attached to the head of the lipid, and

---

[1] *Completeness of examiner's action.* The examiner's action will be complete as to all matters, except that in appropriate circumstances, such as misjoinder of invention, fundamental defects in the application, and the like, the action of the examiner may be limited to such matters before further action is made.

[2] Where the applicant traverses any rejection, the examiner should, if he or she repeats the rejection, take note of the applicant's argument and answer the substance of it.

(c) targeting ligand.

The targeting ligand can be an antibody, a ligand of a receptor, folic acid etc. (column 2, lines 10-57).

Examples of targeting ligand are listed by of Allen et al. in table 1, each of them is specific for a cell type.

The pre-formed liposomes entrap cytotoxic drugs including:

(7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(S)-camptothecin,

7 -(2-(N-isopropylamino)ethyl) - (20S) - camptothecin,

9-aminocamptothecin and 9-nitrocamptothecin (column 3 lines 1-6).

Allen et al. specifically disclose a E-selecting Fab liposomes which target the endothelial cells along the blood vessels (column 14 lines 63-67, fig 5B), and a liposome with a targeting conjugate which bind to breast cancer (column 16).

The targeting capability is due to the targeting ligand instead of the liposome itself.

The person skilled in the art, would not have arrived at the claimed subject-matter because it diverts from the Wang et al. teaching and the same person would not have simplified the Allen et al. teaching by eliminating the targeting ligand. Especially in this case, the skilled person would not have expected lung tropism as shown by the claimed invention.

Even admitting that Allen et al. make an equivalence between a gene and a drug (which applicants do not concede), the combination of Wang et al. and Allen et al does not lead to the claimed invention and does not make the achieved results foreseeable, especially the high intracellular delivery of taxol or of the herein claimed camptothecins and the lung tropism.

Admittedly, Burke teaches stabilization of camptothecins. However, the Examiner did not comment on Applicants' arguments on Burke presented in the previous replies, *see* in particular the ones filed on November 8, 2006 and on June 5, 2008, so applicants do not have the benefit of the examiner's views and interpretation of this reference. Burke et al. are silent on liposomes comprising a compound of formula (II) and, more importantly Burke et al. are silent on **drug delivery** and **organ targeting**.

The person skilled in the art, would not have arrived at the claimed subject-matter because it diverts from Wang et al. teaching and is much simpler than Allen et al. Even admitting that Burke et al. teach camptothecin stabilization by means of liposomes, they do not teach the

liposomes of the present invention and are completely silent on drug delivery and organ targeting. The references, neither alone or in combination make the achieved results foreseeable, especially the high intracellular delivery of taxol or of the herein claimed camptothecins and the lung tropism.

As to Stracher et al., the Examiner admits that carnitine is **part** of the liposome structure, **but carnitine is NOT the liposome.**

Once more, the Examiner failed to comment Applicants' technical arguments in the reply of June 5, 2008 that Stracher et al. disclose carnitines as carriers (*see* abstract), which can be incorporated into liposomes (from column 14 to column 17) useful for diminishing the damage incurred during cardiac ischemia resulting from the activation of calcium activated proteases (column lines 45-56), so applicants do not have the benefit of the examiner's views and interpretation of this reference. **Stracher et al. are silent on tumor.**

Admittedly, Stracher et al. teach carnitine as carrier for drugs, **but specifically to cardiac and skeletal muscle**. However, the Examiner did not comment on Applicants' arguments on Stracher et al. presented in the previous replies, *see* in particular the one filed on June 5, 2008. The Examiner did not comment the fact that Stracher et al. liposomes **may contain carnitine, but are not made of carnitine.**

The person skilled in the art, would not have arrived at the claimed subject-matter because it diverts from Wang et al. teaching, is much simpler than Allen et al. and is quite different from the liposome of Burke et al., moreover, Stracher et al. do not teach liposomes directed to intracellular delivery of tumor cells and does not teach liposomes made of carnitine.

The person of ordinary skill in the art would not have had any reasonable expectation of success in achieving the results of the present invention since this invention goes far from Wang et al. Even modifying the Wang et al. liposomes, but the reference does not suggest in which way, the skilled person would have encountered Allen et al., who teach to use a targeting ligand. Therefore, the skilled person would not have met the present invention, which does not use a targeting ligand as Allen et al. Burke et al. do not use a targeting ligand, but does not provide carnitine liposomes. Moreover, Burke does not teach organ targeting and intracellular delivery, therefore, the skilled person is in a *cul-de-sac.*

This person cannot simplify Allen, without the risk of failing organ targeting and cannot use Burke, who does not direct to organ targeting. Finally, Stracher et al. is of no use to the skilled person, since it deals with skeletal and cardiac muscle and not with tumor, as also shown by the list of drugs in Stracher et al. (all specific for cardiovascular diseases, *see* the entire reference). Stracher et al.'s reference is of no help to the skilled person seeking lung targeting for antitumor drugs. There is no indication in Stracher et al. that skeletal and cardiac muscle targeting for cardiovascular drugs is also fit for lung targeting for antitumor drugs. That carnitines are targeting to cardiac tissue is well-known in the art and also acknowledged by the Applicants, *see* specification page 6, discussing EP 0 279 887.

No reasonable expectation of success is given in the cited references in using the claimed liposomes with those specific acyl and alkyl residues, which indeed are against Wang teaching on efficiency. Moreover, the skilled person is not encouraged by Burke et al. or Allen et al. in changing the molecule forming the liposome with respect to Wang et al. In fact, the only indication for organ targeting is given in Stracher et al., but this is directed to skeletal and cardiac muscle for the transport and delivery of cardiovascular drugs, and not to tumor cells to transport and deliver taxol or camptothecins, in particular to lungs (present claims 71 (now 116) and 94 (now 128) and 116 (now 147)).

Applicants respectfully invite the Examiner to discuss the specific technical points discussed above and to show where in the art **each** specific feature constructing the pending claims is shown and that the same result of the invention is achieved.

Claims 116-124 (now 147-155) are directed to a specific embodiment of the present invention and are non obvious over the cited references, neither alone or in combination.

Wang et al. show that the most efficient liposome to deliver DNA into lung is 4f, namely oleoyl carnitine oleyl ester (*see* Figure 5).

Claim 116 (now 147) does not provide oleoyl/oleyl combination. Oleoyl/oleyl residues are unsaturated acyl/alkyl chains. Claim 116 (now 147) never foresees an unsaturated acyl/alkyl residue. Contrarily, Wang et al. show that even changing just one of the unsaturated oleoyl/oleyl residues with one saturated acyl/alkyl residue (4b oleoyl/stearoyl and 4d myristoyl/myristyl), transfection efficiency in the lungs dramatically falls below efficiency of conventional liposomes DDAB and DOTAP (*see* Figure 5).

Contrarily to every expectation, the liposomes of the present invention are able to deliver significant amounts of antitumor drug to lung tissue (specification, pages 62-63 and Figure 3).

Claims 125-132 (now 156-163) are directed to a specific embodiment of the present invention and are non obvious over the cited references, neither alone or in combination.

The art never recites the specific combination of alky/acyl residue in the carnitine molecule for liposome preparation and does not suggest that these specific liposomes are capable of intracellular delivery of an antitumor drug into tumor cells to the lungs of a subject in need of antitumor treatment.

For both blocks of claims, 116-124 (now 147-155) and 125-132 (now 156-163), the cited art is not inspiring the skilled person.

In fact, Allen clearly teaches that, in order to target a specific organ, the liposome per se is not sufficient; *see* the discussion above. Wang teaches pulmonary tropism, but with liposome having unsaturated acyl/alkyl chains.

Burke does not teach any element for organ tropism and Stracher teaches that L-carnitine derivatives are specific for muscle cells, both skeletal and cardiac.

Clearly, the skilled person cannot find any suggestion to arrive at the elements constructing claims 116 (now 147) or 125 (now 156).

Applicants note that finding an element in the art embedded in one of the dependent claims 117-124 (now 148-155) and 126-132 (now 157-163) does not render obvious claim 116 (now 147) or claim 125 (now 156).

None of the references specifically teach or suggest the claimed subject-matter.

Claims 71-78 (now 116-121) and 86 (now 122)-105 and 107-115 (now 138-146) – 35 USC § 103(a)

Wang et al in combination with Allen, Burke, Stracher et al. and Szoka.

We rely on the previously presented remarks and to the discussion made in the previous section.

The only additional reference to be discussed is Szoka.

Dry powder form is an embodiment of the present invention. Since the invention is not obvious over Wang et al in combination with Allen, Burke and Stracher et al., Szoka does not add any significant contribution.

Respectfully submitted,

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(54) Camptothecin derivatives having antitumor activity

Camptothecin-Derivate mit Antitumor-Wirkung

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Remarks:
The file contains technical information submitted after the application was filed and not included in this specification

EP 1 044 977 B1

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Description

[0001] The present invention relates to compounds having antitumor activity, in particular to new derivatives of camptothecins, processes for their preparation, their use as antitumor drugs and pharmaceutical compositions containing them as active ingredients.

Background of the invention

[0002] Camptothecin is an alkaloid, which was isolated by Wall et al (J. Am. Chem. Soc. 88, 3888-3890 (1966)) for the first time from the tree Camptoteca acuminata, a plant originating from China, of the Nyssaceae family.

[0003] The molecule consists of a pentacyclic structure having a lactone in the ring E, which is essential for cytotoxicity.

[0004] The drug demonstrated a wide spectrum of antitumor activity, in particular against colon tumors, other solid tumors and leukemias, and the first clinical trials were performed in the early 70's. Since Camptothecin (in the following briefly CPT) has low water solubility and in order to prepare clinical trials, the National Cancer Institute (NCI) prepared the sodium salt (NSC100880), which is water-soluble. Clinical trials in phase I and II, were not completed because of the high toxicity showed by the compound (hemorrhagic cystitis, gastrointestinal toxicity, such as nausea, vomit, diarrhoea, and myelosuppression, especially leucopenia and thrombocytopenia.

[0005] In any case, sodium salt showed a lower activity than CPT, because, at pH 7.4, the inactive form (open ring) predominates on the lactone-active one (closed ring), which predominates at pH< 4.0.

[0006] Subsequently, many CPT analogues were synthesised in order to obtain compounds with lower toxicity and higher water solubility. Two drugs are marketed, Irinotecan (CPT-11), marketed with the Trade Mark Camptosar® by Upjohn and Topotecan, marketed with the Trade Mark Hymcamptamin® or Thycantin®, by Smith Kline & Beecham. Other derivatives are in different steps of clinical development in phase II, such as NSC-603071 (9-aminocamptothecin), 9-NC or 9-nitrocamptothecin, an oral prodrug converted in 9-aminocamptothecin, GG-211 (GI 147211), and DX-8591f, the latter being water-soluble. All the derivatives identified to-date contain the parent structure with 5 rings, essential for cytotoxicity. It was demonstrated that modifications on the first ring, such as in the case of the above-mentioned drugs increase water solubility and allow a higher tolerability of the drug.

[0007] Water-soluble Irinotecan was approved for the treatment of many solid tumors and ascites (colon- rectum, skin, stomach, breast, small and non-small cell lung, cervix and ovarian cancer and in non-Hodgkin lymphoma). Moreover, Irinotecan resulted active in solid tumors resistant to Topotecan, vincristine or melphalan and MDR-1 cells resulted marginally resistant to the drug. The active metabolite was identified as the 10-hydroxyderivatives (SN-38), produced by the action of carboxylesterases. CPT-11 showed a good activity using different administration routes, such as intraperitoneal, intravenous, oral (Costin D., Potmhexyl M. Advances in Pharmacol. 29B, 51-72 1994).

[0008] CPT-11 was administered also with cisplatin or etoposide, showing a synergistic effect, thanks to the ability to hinder DNA repair. Also in this case, however, a grade 3 and 4 leucopenia and diarrhoea arose (Sinha B.K. (1995) Topoisomerase inhibitors. Drugs 49, 11-19, 1995).

[0009] Topotecan has a significant oral bioavailability. Oral administration proved to be convenient to reach a prolonged exposition to the drug, without the use of temporary catheters being necessary (Rothenberg M.L. Annals of Oncology 8, 837-855, 1997). Also this water-soluble CPT analogue showed activity against different types of tumors, with different administration routes, intraperitoneal, intravenous, subcutaneous, oral. The more promising results were obtained with Topotecan hydrochloride, intravenous infusion for 5 days, in different tumors such as small and non-small cell lung, ovarian, breast, stomach, liver, prostatae, soft tissue sarcoma, head and neck, oesophagus, resistant colon-rectum, multiform glioblastoma, chronic and acute myelocytic leukemias. However, also in this case, severe side effects occurred, such as neutropenia and thrombocytopenia, whereas gastrointestinal toxicity, such as nausea vomit and diarrhoea were milder.

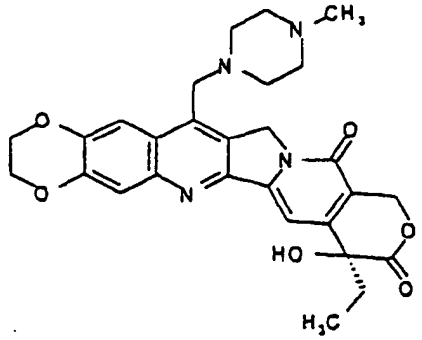
[0010] It was demonstrated that the main transformation -and elimination pathways of the drug comprise lactone hydrolysis and urinary excretion: in fact, lactone form is 50% hydrolysed to open ring, 30 min after infusion. Topotecan crosses hematoencephalic barrier 10 min after infusion (30% in the cerebrospinal fluid with respect to plasma). On the contrary, camptothecin does not cross hematoencephalic barrier in significant amount, probably due to its binding with proteins.

[0011] Clinical development of 9-aminocamptothecin was hampered by its scarce water solubility. Recently, a colloidal dispersion was prepared, which made possible its entry in phase II clinical trial. Prolonged exposition (from 72 hours to 21 days) appeared to be essential to demonstrate antitumor activity, because of its short half-life (Dahut et al., 1994). Responses in patients suffering from not treated colon-rectum, and breast cancer and resistant lymphoma, were noticed. The activity demonstrated against Pgp-positive tumors suggested a lack of cross-resistance against resistant MDR- 1 cells. Once again, bone marrow and gastrointestinal toxicity was observed.

[0012] Lurtotecan is the most water-soluble analogue, with an activity comparable to Topotecan in vitro. Two regimens

were adopted: one 30-min infusion a day for 5 days every 3 weeks and one 72-hours infusion one time every 3 weeks. Responses in patients suffering from, neck, ovarian, breast, liver tumour were observed. Also in this case, hematic toxicity was detected.

[0013] The molecule is the following:



[0014] 9-Nitrocamptothecin is an oral prodrug rapidly converted into 9-aminocamptothecin after administration. Responses were observed in patients suffering from pancreas, ovarian, and breast cancer.

[0015] Notwithstanding the major part of tumour cells is highly sensitive to topoisomerase I inhibitors, due to the high enzyme levels, some tumoral lines result to be resistant. This is due to other mechanisms, rather than the overexpression of MDR1 and MRP (multidrug resistance associated protein) genes and of their products, P (Pgp) glycoprotein and MRP protein, respectively, for which Topotecan or CPT-11 are not very good substrates, (Kawato Y et al J. Pharm. Pharmacol. 45, 444-448, (1993)).

[0016] In fact, it was observed that some resistant tumour cells contain mutant forms of topo I, accordingly the formation of the topo I-DNA complex is damaged or some cells lack in the carboxylesterase activity, necessary for converting CPT-11 in the active metabolite SN-38 and are thus resistant against this drug (Rothenberg, 1997, ibid.).

[0017] Within the drugs used in tumour therapy, the interest in inhibitors of topoisomerase I enzymes is attributed to the following considerations: a) efficacy against tumors naturally resistant to conventional drugs, topoisomerase II inhibitors included; b) the levels of the topo I enzyme remain elevated in all phases of the cycle; c) many tumors express high levels of the target enzyme; d) lack of recognition by the proteins involved in the phenomenon of multidrug resistance (Pgp or MRP) and absence of the detoxifying enzyme-mediated metabolism, associated to the glutathione-dependent system (glutathione peroxidase and glutathione S-transferase) (Gerrits CJH., et al., Brit. J. Cancer 76, 952-962).

[0018] Once potential clinical advantages of topoisomerase I inhibitors are taken into consideration, both in terms of antitumor activity, assayed on a wide range of tumors, and the poor induction of pharmaco-resistance, the present research aims to identify topo I inhibitors with a lower toxicity with respect to the one demonstrated by the drugs on the market or in clinical phase. The factors determining the relative potency of camptothecin analogues include a) intrinsic activity of topoisomerase I inhibition; b) drug mean life; c) interaction with plasma proteins; d) the ratio between the circulating active form (lactone) and the non active one (carboxylate); e) drug sensitivity relative to cell outflow mediated by glycoprotein P or MRP; f) bond stability with topoisomerase I (Rothenberg, 1997, ibid.).

[0019] Among the main adverse effects of Irinotecan and other camptothecins derivatives, myelosuppression and gastrointestinal toxicity, such as diarrhoea and vomit, have been observed. Diarrhoea can have an early or late onset and can be a dose-limiting factor. Vomit and late diarrhoea are induced by many antitumor drugs, while early diarrhoea occurring during or immediately after infusion is almost specific for Irinotecan and some camptothecin derivatives.

[0020] Toxic effects occur mainly in the intestinal tract.

[0021] In order to reduce diarrhoea, CPT-11 was administered in some clinical trials, in combination with loperamide, a synthetic oppioid, agonist of the mu-oppioid enteric receptors (Abigerges, 1994; Abigerges, 1995), as well as with an inhibitor of the enkephalinases (acetorfan) or with ondansetron, an antagonist of the 5-HT3 receptors, or with diphenidramine, an antagonist of H1 receptors.

[0022] To date, the problems connected with the use of camptothecin derivatives as antitumor drugs can be summarised in the following items:

- camptothecin (CPT), and many of its active derivatives have low water solubility;
- the subsequent derivatives are endowed with severe side effects at gastrointestinal and bone marrow level;
- some tumour lines developed resistance against topoisomerase I inhibitors;

- there is the constant search for a better therapeutic index.

[0023] Patent application WO97/31003 discloses derivatives of camptothecins substituted at positions 7, 9 and 10. Position 7 provides the following substitutions: -CN, -CH(CN)-R_4, -CH=C(CN)-R_4, -CH_2-CH=C(CN)-R_4, - C(=NOH) -NH_2, -CH=C(NO_2)-R_4, -CH(CN)-R_5, -CH(CH_2NO_2)-R_5, 5-tetrazolyl, 2-(4,5-dihydroxazolyl), 1,2,4-oxadiazolidin-3-yl-5-one, wherein R_4 is hydrogen, linear or branched alkyl from 1 to 6 carbon atoms, nitrile, carboxyalkoxy. Of these possible compounds, WO97/31003 enables the disclosure only of camptothecin derivatives bearing at position 7 the group -CN and -CH=$C(CN)_2$, with unsubstituted positions 9 and 10.

[0024] Of these compounds, the best one proved to be the 7-nitrile (R_4 = -CN), hereinafter named CPT 83, with cytotoxic activity on non-small cells lung carcinoma (non-SCLC, H-460). This tumour line is intrinsically resistant to cytotoxic therapy and is only moderately responsive to topoisomerase I inhibitors, notwithstanding the overexpression of the target enzyme. CPT 83 is more active than Topotecan, taken as reference compound and on the whole it offers a better pharmacological profile, even in terms of tolerability, then a better therapeutic index.

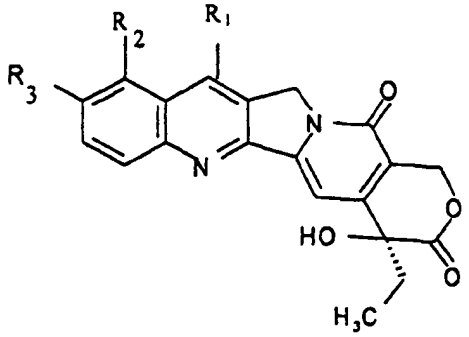
[0025] CPT 83 is prepared through a synthesis route comprising the oxidation of 7-hydroxymethylcamptothecin to camptothecin 7-aldehyde, the transformation of the latter into oxime and final conversion into nitrile.

[0026] The starting compound and the intermediates are disclosed in Sawada et al Chem. Pharm. Bull. **39,** 2574 (1991). This paper makes reference to a patent family with priority of 1981, for example European patent application EP 0056692, published in 1982. In these publications there are disclosed, among others, the compounds camptothecin 7-aldehyde and its oxime. The usefulness of these derivatives is to provide compounds with antitumor activity having low toxicity starting from 7-hydroxymethylcamptothecin. In the paper published on Chem. Pharm. Bull. **39,** 2574 (1991), the authors demonstrate that, with respect to camptothecin, the 7-alkyl and 7-acyloxymethyl derivatives, which were not foreseen in the above mentioned patent application, are the more active compounds on lines of murine leukemia L1210, while lower activity, always with respect to camptothecin, was observed in compounds bearing 7-substitutions with high polar character, such as hydrazones and the oxime - CH(=NOH).

Abstract of the invention

[0027] It has now surprisingly been found that camptothecins bearing an alkyloxime O-substituted at position 7 are endowed with antitumor activity higher than the compound of reference Topotecan. More surprisingly, it has been found that camptothecins bearing an enamino group on position 7, are also endowed with antitumor activity. Said compounds have better therapeutic index.

[0028] Accordingly, it is an object of the present invention compounds of general formula (I):



wherein:

R_1 is a —C(R_5)=N-$O_{(n)}R_4$ group, wherein R_4 is a C_1-C_8 linear or branched alkyl or C_1-C_8 linear or branched alkenyl group or C_3-C_{10} cycloalkyl, or (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl group, or C_6-C_{14} aryl, or (C_6-C_{14}) aryl - (C_1-C_8) linear or branched alkyl group, or a heterocyclic or heterocyclo - (C_1-C_8) linear or branched alkyl group, said heterocyclic group containing at least one heteroatom selected from the group consisting of nitrogen atom, optionally substituted with a (C_1-C_8) alkyl group, and/or oxygen and/or sulfur; said alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, aryl, aryl-alkyl, heterocyclic or heterocyclo-alkyl groups, being optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 alkyl, C_1-C_8 alkoxy, phenyl, cyano, nitro, -NR_6R_7, wherein R_6 and R_7, the same or different between them, are hydrogen, (C_1-C_8) linear or branched

alkyl; the - COOH group or a pharmaceutically acceptable ester thereof; or the - $CONR_8R_9$ group, wherein R_8 and R_9, the same or different between them, are hydrogen, (C_1-C_8) linear or branched alkyl, phenyl; or
R4 is a (C_6-C_{10}) aroyl or (C_6-C_{10}) arylsulfonyl group, optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 linear or branched alkyl, C_1-C_8 linear or branched alkoxy, phenyl, cyano, nitro, -$NR_{10}R_{11}$, wherein R_{10} and R_{11}, the same or different between them are hydrogen, C_1-C_8 linear or branched alkyl;
R_4 is a polyaminoalkyl group of formula —$(CH_2)_m$-NR_{12}-$(CH_2)_p$-NR_{13}-$(CH_2)_q$-NH_2, wherein m, p are an integer from 2 to 6 and q is an integer from 0 to 6, extremes included and R_{12} and R_{13} are a (C_1-C_8) linear or branched alkyl group; or
R_4 is a glycosyl group selected from 6-D-galactosyl, 6-D-glucosyl;
n is the number 1;
R_5 is hydrogen, C_1-C_8 linear or branched alkyl, C_1-C_8 linear or branched alkenyl, C_3-C_{10} cycloalkyl, (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl, C_6-C_{14} aryl, (C_6-C_{14}) aryl - (C_1-C_8) linear or branched alkyl;
R_2 and R_3, the same or different between them are hydrogen, hydroxy, C_1-C_8 linear or branched alkoxy;
their N_1-oxides, their single isomers, in particular the syn and anti isomers of the —$C(R_5)$=N-$O_{(n)}R_4$ group, their possible enantiomers, diastereoisomers and relative mixtures, the pharmaceutically acceptable salts thereof;

[0029] The present invention comprises the use of the compounds of the above-mentioned formula (I) as active ingredients for medicaments, in particular for medicaments useful for the treatment of tumors.

[0030] The present invention comprises pharmaceutical compositions containing compounds of formula (I) as active ingredients, in admixture with pharmaceutically acceptable vehicles and excipients.

[0031] The present invention comprises also processes for the preparation of compounds of formula (I), and the relative key intermediates.

Detailed description of the invention

[0032] Within the scope of the present invention, as examples of C_1-C_8 linear or branched alkyl group, methyl, ethyl, propyl, butyl, pentyl, octyl are meant and their possible isomers, such as for example isopropyl, isobutyl, tert-butyl.

[0033] Examples of C_1-C_8 linear or branched alkenyl group are methylene, ethylidene, vinyl, allyl, propargyl, butylene, pentylene, wherein the carbon-carbon double bond, optionally in the presence of other carbon-carbon unsaturations, can be situated in the different possible positions of the alkyl chain, which can also be branched within the allowed isomery.

[0034] Examples of C_3-C_{10} cycloalkyl group are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, polycyclic groups, such as for example adamantyl.

[0035] Examples of (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl group are cyclopropylmethyl, 2-cyclopropylethyl, 1-cyclopropylethyl, 3-cyclopropylpropyl, 2-cyclopropylpropyl, 1-cyclopropyl-propyl, cyclobutylmethyl, 2-cyclobutylethyl, 1-cyclobutyelthyl, 3-cyclobutylpropyl, 2-cyclobutylpropyl, 1-cyclobutylpropyl, cyclohexylmethyl, 2-cyclohexylethyl, 1-cyclohexylethyl, 3-cyclohexylpropyl, 2-cyclohexylpropyl, 1-cyclohexylpropyl, 5-cyclohexylpentyl, 3-cyclohexylpentyl, 3-methyl-2-cyclohexylbutyl, 1-adamantylethyl, 2-adamantylethyl, adamantylmethyl.

[0036] Examples of (C_6-C_{14}) aryl, or (C_6-C_{14}) aryl - (C_1-C_8) linear or branched alkyl group are phenyl, 1- or 2-naphthyl, anthryl, benzyl, 2-phenylethyl 1-phenylethyl, 3-phenylpropyl, 2-anthrylpropyl, 1-anthrylpropyl, naphthylmethyl, 2-naphthylethyl, 1-naphthylethyl, 3-naphthylpropyl, 2-naphthylpropyl, 1-naphthylpropyl, cyclohexylmethyl, 5-phenylpentyl, 3-phenylpentyl, 2-phenyl-3-methylbutyl.

[0037] Examples of heterocyclic or heterocyclo - (C_1-C_8) linear or branched alkyl group are thienyl, quinolyl, pyridyl, N-methylpyperidinyl, 5-tetrazolyl, 2-(4,5-dihydroxazolyl), 1,2,4-oxadiazolidin-3-yl-5-one, purine and pyrimidine bases, for example uracyl, optionally substituted as shown in the general definitions above-mentioned.

[0038] Examples of (C_6-C_{10}) aroyl groups are benzoyl, naphthoyl.

[0039] Examples of (C_6-C_{10}) arylsulfonyl groups, optionally substituted with an alkyl group, are tosyl, benzenesulfonyl.

[0040] As halogen it is intended fluorine, chlorine, bromine, iodine.

[0041] Examples of substituted groups are pentafluorophenyl, 4-phenylbenzyl, 2,4-difluorobenzyl, 4-aminobutyl, 4-hydroxybutyl, dimethylaminoethyl; p-nitrobenzoyl, p-cyanobenzoyl.

[0042] Examples of polyaminoalkyl group of formula —$(CH_2)_m$-NR_{12}-$(CH_2)_p$-NR_{13}-$(CH_2)_q$-NH_2, wherein m, p are an integer from 2 to 6 and q is an integer from 0 to 6, extremes included and R_{12} and R_{13} are a (C_1-C_8) linear or branched alkyl group, are N-(4-aminobutyl-)-2-aminoethyl, N-(3-aminopropyl)-4-aminobutyl, N-[N-(3-aminopropyl)-N-(4-aminobutyl)]-3-aminopropyl.

[0043] Examples of pharmaceutically acceptable salts are, in case of nitrogen atoms having basic character, the salts with pharmaceutically acceptable acids, both inorganic and organic, such as for example, hydrochloric acid, sul-

furic acid, acetic acid, or, in the case of acid group, such as carboxyl, the salts with pharmaceutically acceptable bases, both inorganic and organic, such as for example, alkaline and alkaline-earth hydroxides, ammonium hydroxide, amines, also heterocyclic ones.

[0044] Preferred compounds of formula (I) are those wherein R_4 is in particular is a C_1-C_8 linear or branched alkyl or C_1-C_8 linear or branched alkenyl group, or C_3-C_{10} cycloalkyl, or (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl, or C_6-C_{14} aryl, or (C_6-C_{14}) aryl - (C1-C_8) linear or branched alkyl, or a heterocyclic or heterocyclo - (C_1-C_8) linear or branched alkyl group, said heterocyclic group containing at least a heteroatom selected from the group consisting of nitrogen atom, optionally substituted with a (C_1-C_8) alkyl group, and/or oxygen and/or sulfur; said alkyl, alkenyl, cycloalkyl, aryl, aryl-alkyl, heterocyclic or heterocyclo-alkyl groups, being optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 alkyl, C_1-C_8 alkoxy, phenyl, cyano, nitro,-NR_6R_7, wherein R_6 and R_7, the same or different between them, are hydrogen, (C_1-C_8) linear or branched alkyl; the -COOH group or a pharmaceutically acceptable ester thereof; or the -$CONR_8R_9$ group, wherein R_8 and R_9, the same or different between them, are hydrogen, (C_1-C_8) linear or branched alkyl, according to the definitions given above by means of example.

[0045] A first group of particularly preferred compounds comprises:

- 7-methoxyiminomethylcamptothecin (CPT 179);
- 7-ethoxyiminomethylcamptothecin;
- 7-isopropoxyiminomethylcamptothecin;
- 7-(2-methylbutoxy)iminomethylcamptothecin;
- 7- t-butoxyiminomethylcamptothecin (CPT 184);
- 7- (4-hydroxybutoxy)iminomethylcamptothecin;
- 7- triphenylmethoxyiminomethylcamptothecin (CPT 192);
- 7-carboxymethoxyiminomethylcamptothecin (CPT 183);
- 7-(2-amino)ethoxyiminomethylcamptothecin (CPT 188);
- 7-(2-N,N-dimethylamino)ethoxyiminomethylcamptothecin(CPT 197);
- 7-allyloxyiminomethylcamptothecin (CPT 195);
- 7-cyclohexyloxyiminomethylcamptothecin;
- 7-cyclohexylmethoxyiminomethylcamptothecin;
- 7-cyclooctyloxyiminomethylcamptothecin;
- 7-cyclooctylmethoxyiminomethylcamptothecin;
- 7-benzyloxyiminomethylcamptothecin (CPT 172);
- 7- [(1-benzyloxyimino)-2-phenylethyl]camptothecin;
- 7- (1-benzyloxyimino)ethylcamptothecin (CPT 186);
- 7- (1-t-butoxyimino)ethylcamptothecin;
- 7-p-nitrobenzyloxyiminomethylcamptothecin (CPT 177);
- 7-p-methylbenzyloxyiminomethylcamptothecin (CPT 178);
- 7-pentafluorobenzyloxyiminomethylcamptothecin (CPT 182);
- 7-p-phenylbenzyloxyiminomethylcamptothecin (CPT 187);
- 7-[2-(2,4-difluorophenyl)ethoxy]iminomethylcamptothecin;
- 7-(4-t-butylbenzyloxy)iminomethylcamptothecin;
- 7-(1-adamantyloxy)iminomethylcamptothecin;
- 7-(1-adamantylmethoxy)iminomethylcamptothecin;
- 7-(2-naphthyloxy)iminomethylcamptothecin;
- 7-(9-anthrylmethoxy)iminomethylcamptothecin;
- 7- (6-uracyl)methoxyiminomethylcamptothecin;
- 7-(4-pyridyl)methoxyiminomethylcamptothecin (CPT 189);
- 7- (2-thienyl)methoxyiminomethylcamptothecin;
- 7-[(N-methyl)-4-piperidinyl]methoxyiminomethylcamptothecin (CPT 190);
- 7-(benzoyloxyiminomethyl)camptothecin (CPT191)
- 7- [(1-hydroxyimino)-2-phenylethyl)camptothecin (CPT 185);

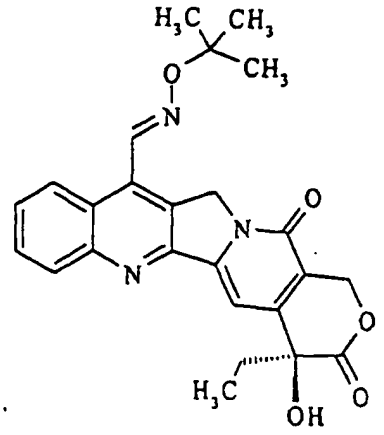
[0046] A second group of particularly preferred compounds comprises:

- 7- [N-(4-aminobutyl)-2-aminoethoxy]iminomethylcamptothecin;
- 7-[N-[N-(3-amino-1-propyl)-4-amino-1-butyl]-3-aminopropoxy]iminomethylcamptothecin;
- 7- (6-D-galattosyloxy)iminomethylcamptothecin;
- 7- (6-D-glucosyloxy)iminomethylcamptothecin;

[0047] In a first preferred embodiment of the invention, compounds of general formula (I) are provided, wherein n is 1, therefore camptothecins 7-oxime, and R_4 is an alkyl or arylalkyl group, as above defined.

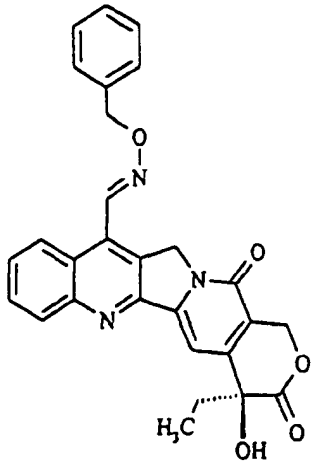
[0048] Among these, the highly preferred compounds are:

7- (t-butoxy)iminomethylcamptothecin (CPT 184) of formula



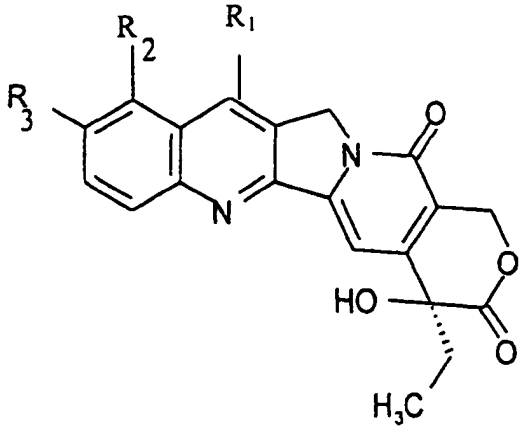
and

7-benzyloxyiminomethylcamptothecin (CPT 172).



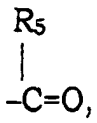
[0049] The compounds of formula (I) can be prepared with different methods according to the nature of the R_4 group and to the presence of the oxygen atom linked to the nitrogen of the 7-iminomethyl group.

[0050] Concerning the compounds of formula (I) wherein n is 1 and R_4 is as above defined, with the exception of aroyl and arylsulfonyl, can be prepared starting from camptothecin 7-aldehyde (formula Ia, R_5 hydrogen) or 7-keto camptothecin (formula Ia, R_5 different from hydrogen).



(Ia)

wherein R_1 is the group



and R_5 is as defined for the formula (I), R_2 and R_3 are as defined in formula (I). The compound of formula (Ia) is reacted with the compound of formula (IIa) $R_4O\text{-}NH_2$, wherein R_4 is as above defined, to give compounds of formula (I), wherein R_1 is the group $—C(R_5)=N\text{-}OR_4$, R_4 is defined as in formula (I), except aroyl and arylsulfonyl. The reaction can be carried out with conventional methods well known to the person skilled in the art, being a normal formation of an oxime. Preferably, the molar ratio between 7-aldehyde or 7-keto camptothecin and hydroxylamine is comprised between 1:3 and 3:1. The salts of the hydroxylamine of interest can also be used. The reaction is carried out in the presence of a base, for example an inorganic base, such as potassium carbonate, or organic, such as triethylamine or diazabicyclononene, using polar solvents, preferably methanol or ethanol and carrying out the reaction at a temperature comprised between room temperature and boiling point of the solvent used, optionally in the presence of dehydrating agents, for example sodium or magnesium sulfate, molecular sieves. If desired it is also possible to carry out the reaction in the presence of a catalyst, for example a Lewis acid.

[0051] Alternatively, the above compounds can be prepared from the oxime of the camptothecin 7-aldehyde (obtained as disclosed in Sawada et al Chem. Pharm. Bull. **39**, 2574 (1991)), or of a 7-keto by reacting with a halide R_4-X, wherein X is preferably iodine, in a polar solvent, for example tetrahydrofurane or alcohols, and in the presence of a base, for example sodium hydride or potassium carbonate.

[0052] As to the compounds of formula (I) wherein n is 1 and R_4 is aroyl or arylsulfonyl, as defined for the formula (I), these can be prepared starting from camptothecin 7-oxime, whose preparation is described in the previous paragraph, with acyl chlorides R_4-COCl, in polar solvents, and in the presence of a base, preferably pyridine, or directly in pyridine, as disclosed by Cho et al. J. Org. Chem. **62**, 2230 (1997).

[0053] The camptothecin 7-aldehyde and the camptothecin 7-oxime are disclosed in the patent application EP 0056692 and in the mentioned Sawada et al Chem. Pharm. Bull. **39**, 2574 (1991),

[0054] N_1-oxides of the compounds of formula (I) are prepared according to well-known methods of oxidation of heteroaromatic nitrogen, preferably by oxidation with acetic or trifluoroacetic acid and hydrogen peroxide, or by reaction with organic peroxyacids (A.Albini and S.Pietra, Heterocyclic N-oxides, CRC, 1991).

[0055] Regarding the various meanings of R_4, present in the different reactives of formula II, these reactives are available in the market, or can be prepared according to well-known methods in literature, which the experts in the field can resort to, completing with their own knowledge of the argument.

[0056] Pharmaceutically acceptable salts are obtained with conventional methods found in the literature, and do not necessitate of further disclosure.

[0057] The compounds disclosed in the present invention show antiproliferative activity, therefore are useful for their therapeutical activity, and posses physico-chemical properties that make them suitable to be formulated in pharma-

ceutical compositions.

[0058] The pharmaceutical compositions comprise at least a compound of formula (I), in an amount such as to produce a significant therapeutical effect, in particular antitumoral effect. The compositions comprised within the present invention are conventional and are obtained with commonly used methods in the pharmaceutical industry. According to the desired administration route, the compositions shall be in solid or liquid form, suitable to the oral, parenteral, intravenous route. The compositions according to the present invention comprise together with the active ingredients at least a pharmaceutically acceptable vehicle or excipient. Formulation co-adjuvants, for example solubilizing, dispersing, suspending, emulsionation agents can be particularly useful.

[0059] The compounds of formula (I) can also be used in combination with other active ingredients, for example other antitumor drugs, both in separate forms, and in a single dose form.

[0060] The compounds according to the present invention are useful as medicaments with antitumor activity, for example in lung tumors, such as the non-small cell lung tumour, tumors of the colon-rectum, prostate, gliomas.

[0061] Cytotoxic activity of the compounds of the present invention was assayed in cell systems of human tumour cells, using the antiproliferative activity test as a method of evaluation of the cytotoxic potential.

[0062] The cell line used is a lung non-small cell carcinoma that belongs to non-small cells hystotype named NCI H460.

[0063] The preferred compounds 7-(t-butoxyiminomethylcamptothecin (CPT 184) and 7-benzyloxyiminomethylcamptothecin (CPT 172) were assayed in comparison with Topotecan (TPT), the reference standard accepted by the persons expert in the field, and with 7-hydroxyiminomethylcamptothecin (CPT 181), disclosed by Sawada et al in Chem. Pharm. Bull. 39(10), 2574-2580, (1991), being the closest structural analogue to the compounds of formula (I) according to the present invention.

[0064] For the *in vivo* studies, the solubilization was carried out in 10% DMSO in bidistilled water, being impossible the solubilization in saline, and the administration for the oral route was carried out at a volume of 10 ml/kg.

Antitumoral activity

[0065] Atimic nu/nu Swiss mice (Charles River, Calco, Italia), ageing 10-12 weeks were used. The animals were maintained in laminar flow rooms, according to the guidelines of the United Kingdom Coordination Committee Cancer Research. Experimental protocols were approved by the Ethical Committee for animal experimentation of Istituto Nazionale per lo Studio e Cura dei Tumori.

[0066] Tumour fragments of about 2x2x2 mm coming from mice to which were inoculated s.c. 10^6 cells NCI H460 mouse, were implanted s.c. bilaterally in groups of 5 mice each.

[0067] The animals were treated with the compounds when the tumour began to be palpable, according to the following scheme:

- CPT172 (8 mg/kg, po) q4dx4
- CPT172 (16 mg/kg, po) q4dx4
- CPT172 (24 mg/kg, po) q4dx4
- CPT172 (2 mg/kg, po) qdx5x10w
- CPT181 (15 mg/kg, po) q4dx4
- CPT181 (25 mg/kg, po) q4dx4
- CPT184 (2 mg/kg, po) q4dx4
- CPT184 (5 mg/kg, po) q10dx6
- Topotecan (15 mg/kg, po) q4dx4
- Topotecan (10 mg/kg, po) q4dx4

[0068] Twice a week, using a Vernier caliper, the width, minimum diameter (l), length and maximum diameter (L) of the tumors were measured, in mm. The tumour volume (mm^3) was calculated according to the formula $l^2xL/2$. Efficacy of the molecule was evaluated as TVI percent of the treated group versus the control group according to the formula TVI%=100-(T/Cx100), wherein T is the mean value of the tumour volume of the treated group and C of control one. A compound is considered active when TVI% $\geq$ 50.

[0069] The following Table 1 reports the experimental results.

TABLE 1

Antitumoral activity of the camptothecin analogues in the treatment of the lung carcinoma NCI H460

Compound	Dose (mg/kg, p.o.)	Treatment scheme	Efficacy (TVI%)	Toxicity	
				Lethality	Body weight loss (%)
CPT172	8	q4dx4	77		
	16	q4dx4	88		
	24	q4dx4	97	0/4	6
	2	qdx5x10w	90	0/3	0
CPT181	15	q4dx4	40	0/4	0
	25	q4dx4	70	0/4	0
CPT184	2	q4dx4	100	0/5	0
	5	q10dx6	99	0/4	9
TPT	15	q4dx4	94	0/4	0
	15	q4dx4	89	0/5	10
	10	q4dx4	64	0/5	0

TVI% was evaluated 5-10 days after the last treatment.

[0070] CPT172 demonstrated an antitumoral efficacy at different doses and at different treatment schemes; CPT184 revealed to be a very active compound at low doses and at different treatment schemes, accordingly, both compounds are two particularly promising molecules for clinical application.

[0071] Further advantages of these molecules can be identified in the wide interval of effective doses, indicating an increase of therapeutic index and a higher handling in the therapeutical use, in particular if a prolonged administration in the time is foreseen, above all in the injectable formulations, with the use of variable schemes and doses. For such uses, compound CPT 172 appears more favourable in relation to the reduced toxicity.

[0072] An important drawback of conventional camptothecins is the reversibility of the their bond in the ternary complex (drug-DNA-enzyme). This reversibility affects drug efficacy, as it does not allow the transformation of the single strand DNA cleavage into double strand DNA cleavage during DNA synthesis.

[0073] Table 2 below shows the persistence of DNA cleavage to a selected number of in-vitro cleavage sites. After 20 minutes of incubation of the drug in the reaction mixture containing labelled DNA and the purified enzyme, sodium chloride (0,6 M) was added with the scope of assisting the dissociation of the ternary complex. The result, shown in the table as percentage of DNA cleavage persistence at the sites, examined after about 10 minutes, is an indication of an almost complete reversibility of the cleavages in the case of camptothecin and Topotecan and a marked persistence in the case of CPT 172 and CPT 184.

TABLE 2

PERSISTENCE OF DNA CLEAVAGE STIMULATED BY CAMPTOTHECINS AND MEDIATED BY TOPOISOMERASE I ON SELECTED SITES	
DRUG (10 μm)	PERSISTENCE (%)
Camptothecin	16
Topotecan	16
CPT 181	28
CPT 184	72
CPT 172	80

[0074] The advantage offered by the compounds according, to the present invention is evident in overcoming the limit of reversibility of the ternary complex with respect to the state of the art.

[0075] The following examples further illustrate the invention.

EXAMPLE 1

7-benzyloxyiminomethylcamptothecin (CPT 172)

[0076] 500 mg (1.33 mmoles) of 7-formylcamptothecin were dissolved into 100 ml of ethanol. 15 ml of pyridine and 638 mg (4 mmoles) of O-benzylhydroxylamine hydrochloride were added and were left for 5 hours to reflux. The solvent was evaporated under vacuum and the residue so obtained was purified by means of flash chromatography on silica gel using a mixture of hexane/ethyl acetate 4/6 as eluant.

Yield 65%

m.p.: 200°-205°C dec.

[0077] The obtained product is constituted by an about 8:2 mixture of the two syn and anti isomers (isomer A: Rf 0.32; isomer B, Rf: 0.19 on silica gel Merck 60 F_{254}, eluant hexane/ethyl acetate 3/7).

[0078] HPLC: the analyses were carried out on an instrument equipped with a quaternary pump (HP 1050) with Rheodyne injector (20 μl loop) and with a diode array detector (HP 1050) controlled by a software HPLC-ChemStation. Spectra acquisition was made from 200 to 600 nm and the chromatograms were registered at 360 and 400 nm.

[0079] A C18 reverse phase column (Rainin C18; 25x0.4 cm, Varian) was used with an RP18 precolumn. The analysis was carried out with a linear elution gradient, starting from acetonitrile:water 30:70 to acetonitrile 100% in 20 min, with 1 ml/min flow. Retention times were: 12.51 min for isomer B and 14.48 for isomer A.

^{1}H-NMR (300 MHz; DMSO-d_6):

δ: 0.88 (t, H3-18A+H3-18B), 1.87 (m, H2-19A+H2-19B), 5.18 (s, H2-5B), 5.21 (s, H2-PhB), 5.30 (H2-PhA), 5.40 (s, H2-5A), 5.45 (s, H2-17A+H2-17B), 6.53 (s, OHA+OHB), 7.3-7.6 (m, ArA+ ArB+H-14A+H-14B), 7.75 (m, H-11A+H-11B). 7.85-7.95 (m, H10A+H-10B), 7.98 (dd, H-12B), 8.18-8.27 (m, H-12A+H9-B), 8.45 (s, CH=NB), 8.59 (dd, H-9A), 9.38 (s, CH=N A).

Mass m/z 481 (M^+ 100) 374
(30)330(70)300(30)273(20)243(20)91(34).

EXAMPLE 2

7-t-butoxyiminomethylcamptothecin (CPT 184)

[0080] 400 mg (1.06 mmoles) of 7-formylcamptothecin were dissolved in 80 ml of ethanol. 12 ml of pyridine and 400 mg (3.18 mmoles) of O-t-butylhydroxylamine hydrochloride were added and left for 4 hours to reflux. The solvent was evaporated under vacuum and the residue so obtained was purified by means of flash chromatography on silica gel using a mixture of hexane/ethyl acetate 4/6 as eluant. 322 mg (0.72 mmoles) of a yellow solid were obtained.
Yield 68%
m.p.: 250°C dec.

[0081] The obtained product is constituted by an about 8:2 mixture of the two syn and anti isomers (isomer A: Rf 0.31; isomer B. Rf: 0.24 on silica gel Merck 60 F_{254}, eluant hexane/ethyl acetate 3/7).

[0082] HPLC: the analyses were carried out on an instrument equipped with a quaternary pump (HP 1050) with Rheodyne injector (20 µl loop) and with a diode array detector (HP 1050) controlled by a software HPLC-ChemStation. Spectra acquisition was made from 200 to 600 nm and the chromatograms were registered at 360 and 400 nm.

[0083] A C18 reverse phase column (Rainin C18; 25x0.4 cm, Varian) was used with an RP18 precolumn. The analysis was carried out with a linear elution gradient, starting from acetonitrile:water 30:70 to acetonitrile 100% in 20 min, with 1 ml/min flow. Retention times were: 12.92 min for isomer B and 14.61 for isomer A.
^{1}H-NMR (300 MHz; DMSO-d_6):
δ: 0.88 (t, H3-18A+H3-18B), 1.30 (s, t-but.B), 1.47 (s, t-but.A) 1.87 (m, H2-19A+H2-19B) 5.18 (s, H2-5 B), 5.37 (H2-5 A), 5.42 (s, H2-17A+H2-17B), 6.54 (s, OHA+OHB), 7.35 (s, H-14A). 7.36 (s, H-14B) 7.69-7.83 (m, H-11A+H-11B), 7.85-7.98 (m, H-10A+H-10B), 8.07 (dd, H-9B), 8.16-8.27 (m, H-9A+H-12B) 8.40 (s, CHB), 8.62 (dd, H-12A), 9.31 (s, CHA).
Mass m/z 448 (M^+ 28) 391 (40)374(100)362(40)330(34)57(17).

[0084] According to the same procedure the following compounds were prepared:

7-p-nitrobenzyloxyiminomethylcamptothecin (CPT 177);
7-p-methylbenzyloxyiminomethylcamptothecin (CPT 178) m.p. 203°C dec.
7-methoxyiminomethylcamptothecin (CPT 179) m.p. 230°C dec.
7-pentafluorobenzyloxyiminomethylcamptothecin (CPT 182) m.p. 200°C dec.
7-carboxymethoxyiminomethylcamptothecin (CPT 183);
7-p-phenylbenzyloxyiminomethylcamptothecin (CPT 187) m.p. 200-202°C dec.
7-(2-amino)ethoxyiminomethylcamptothecin (CPT 188); m.p. 220°C dec.
7-(4-pyridyl)methoxyiminomethylcamptothecin (CPT 189) m.p. 220°C dec, mass m/z M^+482
7-[(N-methyl)-4-pyperidinyl]methoxyiminomethylcamptothecin (CPT 190) m.p. 185-190°C dec, mass m/z M^+502
7-ethoxyiminomethylcamptothecin;
7-isopropyloxyimiminomethylcamptothecin
7-(2-methylbutoxy)iminomethylcamptothecin;
7-cyclohexyloxyiminomethylcamptothecin;
7-cyclohexylmethoxyiminomethylcamptothecin;
7-cyclooctyloxyiminomethylcamptothecin;
7-cyclooctylmethoxyiminomethylcamptothecin;
7-(1-adamantyloxy)iminomethylcamptothecin;
7-(1-adamantylmethoxy)iminomethylcamptothecin;
7-(2-naphthyloxy)iminomethylcamptothecin;
7-(9-anthrylmethoxy)iminomethylcamptothecin;
7-[2-(2,4-difluorophenyl)ethoxy]iminomethylcamptothecin;
7-(4-t-butylbenzyloxy)iminomethylcamptothecin;
7- triphenylmethoxyiminomethylcamptothecin (CPT 192) m.p. 140°C dec;
7- (2-N,N-dimethylaminoethoxy)iminomethylcamptothecin (CPT 197);
7- [N-(4-aminobutyl)-2-aminoethoxy]iminomethylcamptothecin;
7-[N-[N-(3-amino-1-propyl)-4-amino-1-butyl]-3-aminopropoxy]iminomethylcamptothecin;
7- (6-uracyl)methoxyiminomethylcamptothecin;
7- (4-hydroxybutoxy)iminomethylcamptothecin;
7- (2-thienyl)methoxyiminomethylcamptothecin;

7- (4-thiazolyl)methoxyiminomethylcamptothecin;
7- (6-D-galactosyloxy)iminomethylcamptothecin;
7- (6-D-glucosyloxy)iminomethylcamptothecin;
7- (1-benzyloxyimino)ethylcamptothecin (CPT 186);
7- [1(-t-butoxyimino)ethyl]camptothecin;

EXAMPLE 3

7-benzoylcamptothecin (CPT 170)

[0085] Sulfuric acid conc. (0.17 ml) and benzaldehyde (304 mg, 2.87 mmoles) were dropped into a suspension of camptothecin (200 mg, 0.57 mmoles) in CH_3COOH (0.8 ml) and water (0.8 ml). The reaction mixture was cooled down to 0°C and 80% t-butyl peroxide (128 mg, 1.14 mmol) and a solution of $FeSO_4$ (317 mg, 1.14 mmol) in water (0,56 ml) were subsequently added.

[0086] After stirring overnight at room temperature, water was added, a precipitate was obtained, which was filtered under vacuum. The mother liquors were extracted with methylene chloride (3 times); the organic phases were dried over Na_2SO_4, filtered and evaporated under vacuum. The solid thus obtained was gathered with the precipitate, which was separated before. The product was purified by means of flash chromatography on silica gel using as eluant a mixture of methylene chloride/methanol 98/2. 90 mg (0.2 mmoles) of product were obtained.
Yield 35%
^{1}H-NMR (300 MHz; DMSO-d_6):
δ: 0.9 (t, 3H H3-18), 1.85 (m, 2H,H2-19), 5 (s, 2H,H2-5), 5.4(2H,H2-5), 5.4 (s, 2H H2-17), 6.6(s, -1H OH), 7.4 (s1H, H14), 7.55-7.85 (m, 5H,H1-10,H-11,3Ar), 7.95-8 (m, 3H-H12 2Ar), 8.3 (dd, 1H-H-9).

EXAMPLE 4

7-[α-(hydroxyimino)benzyl]camptothecin (CPT 185);

[0087] A solution of 7-benzoylcamptothecin (50 mg, 0.11 mmoles), hydroxylamine hydrochloride (24 mg, 0.33 mmoles), pyridine (1.4 ml) in 10 ml of ethanol was prepared and left 24 hours to reflux. The solvent was eliminated under vacuum. The product was purified by means of flash chromatography on silica gel using a mixture of methylene chloride/methanol 98/2 as eluant. 25 mg of a yellow solid were obtained.
Yield 48%
[0088] The obtained product is constituted of a mixture of the two syn and anti isomers (isomer A: Rf 0.35; isomer B, Rf: 0.31 on silica gel Merck 60 F_{254}, eluant methylene chloride/methanol 95/5).
^{1}H-NMR (300 MHz; DMSO-d_6):
δ: 0.9(t, H3-18A+H3-18B), 1.86 (m, H2-19A+H2-19B) 4.8 (m, H2-5 A+H2-5 B), 5.85 (s, H2-17A+H2-17B), 6.55 (s, -OH B), 7.60 (s OH A), 7.35-7.55 (m, Ar A+Ar B+ H-10A+H-10B+ H-11A+H-11B+H-14A+H-14B) 7.6-7.7 (m, H-12A+H-12B)

EXAMPLE 5

7-(benzoyloxyiminomethylmethyl)camptothecin (CPT191)

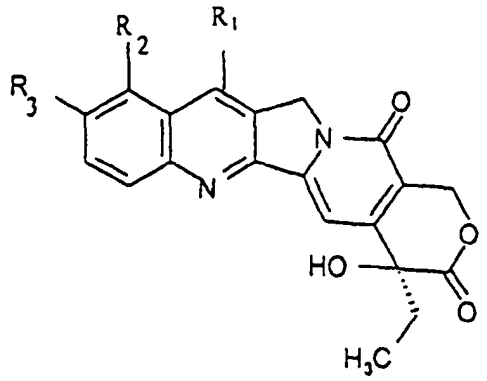
[0089] A solution of benzoyl chloride (0.16 ml, 1.4 mmoles) in 5 ml of pyridine was prepared and 500 mg (1.3 mmoles) of 7-hydroxyiminomethylcamptothecin were added and left overnight under stirring at room temperature. After evaporating pyridine under vacuum, a solution of sodium bicarbonate was added and it was extracted three times with methylene chloride. After drying with sodium sulfate and filtration, the solvent was evaporated off. The product was purified by means of flash chromatography on silica gel using a mixture of methylene chloride/methanol 98/2 as eluant. 200 mg (0.04 mmoles) of a yellow solid were obtained.
Yield 32%.
m.p.: 210°C dec
^{1}H-NMR (300 MHz; DMSO-d_6):
δ: 0.8(t, H3-), 1.8 (m, H2) 5.45 (s, H2-5), 5.55 (s, H2-17), 6.6 (s, 1H - OH), 7.3 (s 1H, H-14), 7.75-8 (m, 5H H-10+H-11+3Ar) 8.25 (m, 2H, 2Ar) 8.3 (dd, 1H, H-12) 8.75 (dd, 1H, H-9), 10.05 (s, 1H, CH=N).
[0090] Following the same procedure the following compounds were prepared:

7-p-nitrobenzoyloxyiminomethylcamptothecin
7-p-cyanobenzoyloxyiminomethylcamptothecin

7-p-tolylsulfonyloxyiminomethylcamptothecin

Claims

1. Compounds of formula (I)



wherein:

R_1 is a —$C(R_5)$=N-$O_{(n)}R_4$ group, wherein R_4 is a C_1-C_8 linear or branched alkyl or C_1-C_8 linear or branched alkenyl group or C_3-C_{10} cycloalkyl, or (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl group, or C_6-C_{14} aryl, or (C_6-C_{14}) aryl - (C_1-C_8) linear or branched alkyl group, or a heterocyclic or heterocyclo - (C_1-C_8) linear or branched alkyl group, said heterocyclic group containing at least a heteroatom selected from the group consisting of nitrogen atom, optionally substituted with a (C_1-C_8) alkyl group, and/or oxygen and/or sulfur; said alkyl, alkenyl, cycloalkyl, cycloalkylalkyl, aryl, aryl-alkyl, heterocyclic or heterocyclo-alkyl groups, being optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 alkyl, C_1-C_8 alkoxy, phenyl, cyano, nitro, -NR_6R_7, wherein R_6 and R_7, the same or different between them, are hydrogen, (C_1-C_8) linear or branched alkyl; the -COOH group or a pharmaceutically acceptable ester thereof; or the -$CONR_8R_9$ group, wherein R_8 and R_9, the same or different between them, are hydrogen, (C_1-C_8) linear or branched alkyl, phenyl; or
R4 is a (C_6-C_{10}) aroyl or (C_6-C_{10}) arylsulfonyl group, optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 linear or branched alkyl, C_1-C_8 linear or branched alkoxy, phenyl, cyano, nitro, -$NR_{10}R_{11}$, wherein R_{10} and R_{11}, the same or different between them are hydrogen, C_1-C_8 linear or branched alkyl;
R_4 is a polyaminoalkyl group of formula —$(CH_2)_m$-NR_{12}-$(CH_2)_p$-NR_{13}-$(CH_2)_q$-NH_2, wherein m, p are an integer from 2 to 6 and q is an integer from 0 to 6, extremes included and R_{12} and R_{13} are a (C_1-C_8) linear or branched alkyl group; or
R_4 is a glycosyl group selected from 6-D-galactosyl, 6-D-glucosyl;
n is the number 1;
R_5 is hydrogen, C_1-C_8 linear or branched alkyl, , C_1-C_8 linear or branched alkenyl, , C_3-C_{10} cycloalkyl, (C_3-C_{10}) cycloalkyl - (C_1-C_8) linear or branched alkyl, C_6-C_{14} aryl, (C_6-C_{14}) aryl - (C_1-C_8) linear or branched alkyl;
R_2 and R_3, the same or different between them are hydrogen, hydroxy, C_1-C_8 linear or branched alkoxy;
their N_1-oxides, their single isomers, in particular the syn and anti isomers of the —$C(R_5)$=N-$O_{(n)}R_4$ group, their possible enantiomers, diastereoisomers and relative admixtures the pharmaceutically acceptable salts thereof.

2. Compounds according to claim 1, selected from the group consisting of:

- 7-methoxyiminomethylcamptothecin;
- 7-ethoxyiminomethylcamptothecin;
- 7-isopropoxyiminomethylcamptothecin;
- 7-(2-methylbutoxy)iminomethylcamptothecin;
- 7- t-butoxyiminomethylcamptothecin;

- 7- (4-hydroxybutoxy)iminomethylcamptothecin;
- 7- triphenylmethoxyiminomethylcamptothecin;
- 7-carboxymethoxyiminomethylcamptothecin;
- 7-(2-amino)ethoxyiminomethylcamptothecin;
- 7- (2-N,N-dimethylamino)ethoxyiminomethylcamptothecin;
- 7-allyloxyiminomethylcamptothecin;
- 7-cyclohexyloxyiminomethylcamptothecin;
- 7-cyclohexylmethoxyiminomethylcamptothecin;
- 7-cyclooctyloxyiminomethylcamptothecin;
- 7-cyclooctylmethoxyiminomethylcamptothecin;
- 7-benzyloxyiminomethylcamptothecin;
- 7- [(1-benzyloxyimino)-2-phenylethyl]camptothecin;
- 7- (1 -benzyloxyimino)ethylcamptothecin;
- 7- (1-t-butoxyimino)ethylcamptothecin
- 7-p-nitrobenzyloxyiminomethylcamptothecin;
- 7-p-methylbenzyloxyiminomethylcamptothecin;
- 7-pentafluorobenzyloxyiminomethylcamptothecin;
- 7-p-phenylbenzyloxyiminomethylcamptothecin;
- 7-[2-(2,4-difluorophenyl)ethoxy]iminomethylcamptothecin;
- 7-(4-t-butylbenzyloxy)iminomethylcamptothecin;
- 7-(1-adamantyloxy)iminomethylcamptothecin;
- 7-(1-adamantylmethoxy)iminomethylcamptothecin;
- 7-(2-naphthyloxy)iminomethylcamptothecin;
- 7-(9-anthrylmethoxy)iminomethylcamptothecin;
- 7- (6-uracyl)methoxyiminomethylcamptothecin;
- 7-(4-pyridyl)methoxyiminomethylcamptothecin;
- 7- (2-thienyl)methoxyiminomethylcamptothecin;
- 7-[(N-methyl)-4-piperidinil]methoxyiminomethylcamptothecin;
- 7-(benzoyloxyiininomethylmethyl)camptothecin;
- 7- [(1-hydroxyimino)-2-phenylethyl)camptothecin.

3. Compounds according to claim 1, selected from the group consisting of:

- 7-[N-(4-aminobutyl)-2-aminoethoxy]iminomethylcamptothecin;
- 7-[N-[N-(3-amino-1-propyl)-4-amino-1-butyl]-3-aminopropyl)iminomethyl-camptothecin.

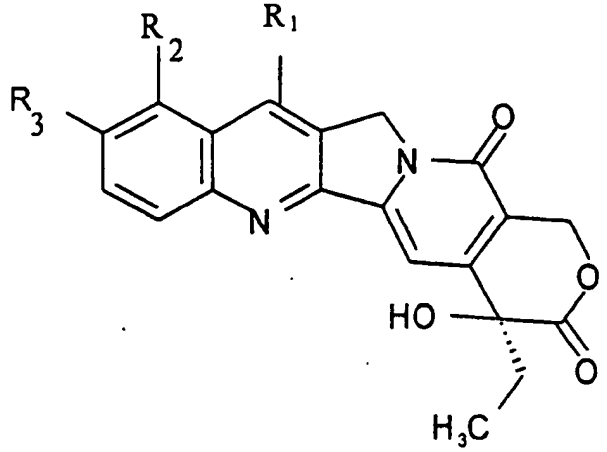
4. Compounds according to claim 1, selected from the group consisting of:

- 7- (6-D-galactosyloxy)iminomethylcamptothecin;
- 7- (6-D-glucosyloxy)iminomethylcamptothecin.

5. Compound according to claim 2, which is 7-(t-butoxy) iminomethylcamptothecin.

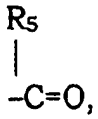
6. Compound according to claim 2, which is 7-benzyloxyiminomethylcamptothecin.

7. A process for the preparation of the compounds of claim 1, wherein n is 1 and R_4 is as above defined, with the exception of aroyl and arylsulfonyl, comprising the reaction of a compound of formula (Ia)



(Ia)

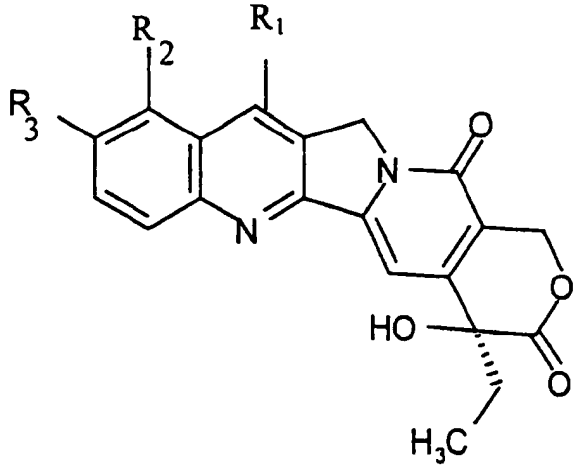
wherein R_1 is the group



and R_5 is as defined for the formula (I), R_2 and R_3 are as defined in formula (I), with the compound of formula (IIa) $R_4O\text{-}NH_2$, wherein R_4 is as above defined, to give compounds of formula (I) wherein R_1 is the group —$C(R_5)$ =N-OR_4, R_4 is defined as in formula (I), except aroyl and arylsulfonyl.

8. Process according to claim 7, wherein the molar ratio between compound of formula (Ia) and compound of formula (IIa) is comprised between 1:3 and 3:1.

9. Process for the preparation of the compounds of the claim 1, wherein n is 1 and R_4 is as above defined, with the exception of aroyl and arylsulfonyl, that comprises the reaction of a compound of formula (Ia)

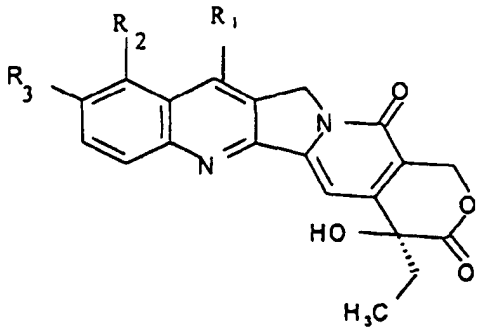


(Ia)

wherein R_1 is the group

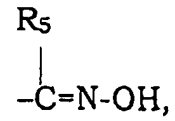
and R_5 is as defined for formula (I), R_2 and R_3 are as defined in formula (I), with a halide R_4-X, wherein X is a halogen and R_4 is as above defined, to give compounds of formula (I), wherein R_1 is the group — $C(R_5)$=N-OR_4, R_4 is defined as in formula (I), except aroyl and arylsulfonyl.

10. Process for the preparation of the compounds of claim 1, wherein n is 1 and R_4 is aroyl or arylsulfonyl, comprising the reaction of a compound of formula (Ia)



(Ia)

wherein R_1 is the group



and R_5 is as defined for formula (I), R_2 and R_3 are as defined in formula (I), with an acyl chloride R_4-COCl, wherein R_4 is aroyl or arylsulfonyl as above, to give compounds of formula (I), wherein R_1 is the group — $C(R_5)$=N-OR_4, R_4 is aroyl or arylsulfonyl.

11. Compounds according to any one of claims 1-6, as medicaments.

12. Pharmaceutical composition comprising a therapeutically effective amount of at least a compound of claims 1-6, in admixture with pharmaceutically acceptable vehicles and excipients.

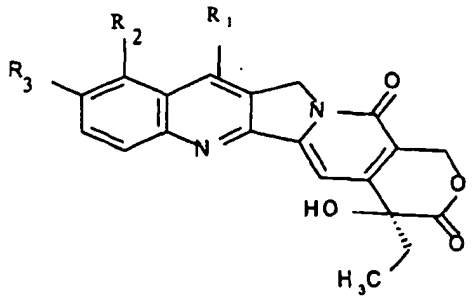
13. Pharmaceutical composition comprising a therapeutically effective amount of at least a compound of claims 1-6, in admixture with pharmaceutically acceptable vehicles and excipients and optionally in combination with other active ingredients.

14. Pharmaceutical composition according to claim 13 wherein said other active ingredient is an antitumoral.

15. Use of a compound of claims 1-6, for the preparation of a medicament useful for the treatment of tumors.

16. Use according to claim 15, wherein said tumour is a lung non-small cell carcinoma.

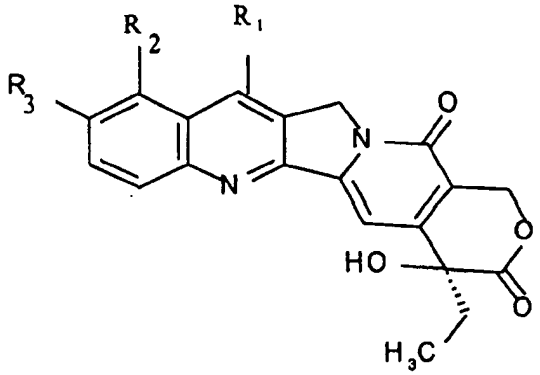
17. Use of compounds of formula (Ia)



wherein:

R_1 is a group —$C(R_5)$=N-OR_4, wherein R_4 is hydrogen; or a group (C_6-C_{10}) aroyl or arylsulfonyl, optionally substituted with one or more groups selected from the group consisting of: halogen, hydroxy, C_1-C_8 linear or branched alkyl, C_1-C_8 linear or branched alkoxy, phenyl, cyano, nitro, -$NR_{10}R_{11}$, wherein R_{10} and R_{11}, the same or different between them, are hydrogen, C_1-C_8 linear or branched alkyl;
R_2 and R_3, the same or different between them are hydrogen, hydroxy, C_1-C_8 linear or branched alkoxy; as intermediates in the process of claim 10.

18. Use of compounds of formula (Ia)

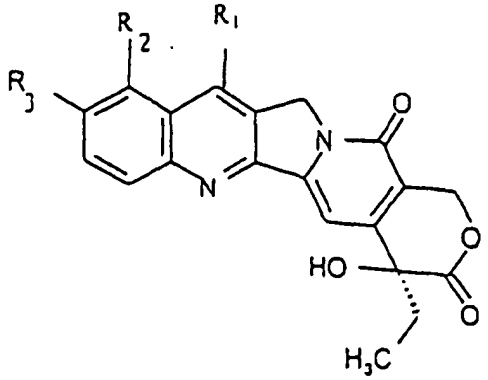


(Ia)

wherein R_1 is the group —$C(R_5)$=O, and R_5 is as defined for the formula (I), R_2 and R_3 are as defined in formula (I), as intermediates in the process of the claim 7.

Patentansprüche

1. Verbindungen der Formel (I)



worin R_1 eine —$C(R_5)$=N-$O_{(n)}R_4$-Gruppe ist, worin R_4 eine C_1-C_8- lineare oder verzweigte Alkyl-Gruppe oder lineare oder verzweigte C_1-C_8-Alkenyl-Gruppe oder C_3-C_{10}-Cycloalkyl oder (C_3-C_{10})-Cycloalkyl-(C_{1-8})-lineare oder verzweigte Alkyl-Gruppe oder C_6-C_{14}-Aryl oder (C_6-C_{14})-Aryl- (C_1-C_8)- lineare oder verzweigte Alkyl-Gruppe oder heterocyclische oder Heterocyclo-(C_{1-8})-lineare oder verzweigte Alkyl-Gruppe ist, wobei die heterocyclische Gruppe zumindest ein Heteroatom enthält, ausgewählt aus der Gruppe, bestehend aus Stickstoffatom, wahlweise substituiert mit einer (C_{1-8})-Alkyl-Gruppe und/oder Sauerstoff und/oder Schwefel; wobei die Alkyl-, Alkenyl-, Cycloalkyl-, Cycloalkylalkyl-, Aryl-, Arylalkyl-, heterocyclische oder Heterocyclo-alkyl-Gruppen wahlweise mit einer oder mehreren Gruppen substituiert sind, ausgewählt aus der Gruppe, bestehend aus: Halogen, Hydroxy, C_1-C_8-Alkyl, C_1-C_8-Alkoxy, Phenyl, Cyano, Nitro, -NR_6R_7, worin R_6 und R_7, die gleich oder verschieden sind, Wasserstoff, (C_{1-8})- lineares oder verzweigtes Alkyl sind; die -COOH-Gruppe oder ein pharmazeutisch akzeptabler Ester davon; oder die -$CONR_8R_9$-Gruppe, worin R_8 und R_9, die gleich oder verschieden sind, Wasserstoff, (C_1-C_8)- lineares oder verzweigtes Alkyl, Phenyl sind; oder

R_4 eine (C_6-C_{10})-Aroyl- oder (C_6-C_{10})-Arylsulfonyl-Gruppe ist, die wahlweise mit einer oder mehreren Gruppen substituiert ist, ausgewählt aus der Gruppe, bestehend aus Halogen, Hydroxy, lineares oder verzweigtes C_1-C_8-Alkyl, lineares oder verzweigtes C_1-C_8-Alkoxy, Phenyl, Cyano, Nitro, -$NR_{10}R_{11}$, worin R_{10} und R_{11}, die gleich oder verschieden sein können, Wasserstoff, C_1-C_8- lineares oder verzweigtes Alkyl sind;

R_4 eine Polyaminoalkyl-Gruppe der Formel -$(CH_2)_m$-NR_{12}-$(CH_2)_p$-NR_{13}-$(CH_2)_q$-NH_2 ist, worin m, p eine ganze Zahl von 2 bis 6 sind und q eine ganze Zahl von 0 bis 6 ist, wobei die Grenzen eingeschlossen sind, und R_{12} und R_{13} eine lineare oder verzweigte (C_{1-8})-Alkyl-Gruppe sind; oder

R_4 eine Glycosyl-Gruppe ist, ausgewählt aus 6-D-Galactosyl, 6-D-Glucosyl;

n die Zahl 1 ist;

R_5 Wasserstoff, lineares oder verzweigtes C_1-C_8-Alkyl, lineares oder verzweigtes C_1-C_8-Alkenyl, C_3-C_{10}-Cycloalkyl, (C_3-C_{10})-Cycloalkyl-(C_1-C_8)-lineares oder verzweigtes Alkyl, C_6-C_{14}-Aryl, (C_6-C_{14})-Aryl-(C_{1-8})- lineares oder verzweigtes Alkyl ist;

R_2 und R_3, die gleich oder verschieden sein können, Wasserstoff, Hydroxy, lineares oder verzweigtes C_1-C_8-Alkoxy sind;

deren N_1-Oxide, deren einzelnen Isomere, insbesondere die syn- und anti-Isomeren der -$C(R_5)$=N-$O_{(n)}R_4$-Gruppe, deren möglichen Enantiomeren, Diastereoisomeren und jeweiligen Mischungen, die pharmazeutisch akzeptablen Salze davon.

2. Verbindungen nach Anspruch 1, ausgewählt aus der Gruppe, bestehend aus:

- 7-Methoxyiminomethylcamptothecin;
- 7-Ethoxyiminomethylcamptothecin;
- 7-Isopropoxyiminomethylcamptothecin;
- 7-(2-Methylbutoxy)iminomethylcamptothecin;
- 7-t-Butoxyiminomethylcamptothecin;
- 7-(4-Hydroxybutoxy)iminomethylcamptothecin;
- 7-Triphenylmethoxyiminomethylcamptothecin;
- 7-Carboxymethoxyiminomethylcamptothecin;
- 7-(2-Amino)ethoxyiminomethylcamptothecin;
- 7-(2-N,N-Dimethylamino)-ethoxyiminomethylcamptothecin;

- 7-Allyloxyiminomethylcamptothecin;
- 7-Cyclohexyloxyiminomethylcamptothecin;
- 7-Cyclohexylmethoxyiminomethylcamptothecin;
- 7-Cyclooctyloxyiminomethylcamptothecin;
- 7-Cyclooctylmethoxyiminomethylcamptothecin;
- 7-Benzyloxyiminomethylcamptothecin;
- 7-[(1-Benzyloxyimino)-2-phenylethyl]camptothecin;
- 7-(1-Benzyloxyimino)ethylcamptothecin;
- 7-(1-t-Butoxyimino)ethylcamptothecin;
- 7-p-Nitrobenzyloxyiminomethylcamptothecin;
- 7-p-Methylbenzyloxyiminomethylcamptothecin;
- 7-Pentafluorbenzyloxyiminomethylcamptothecin;
- 7-p-Phenylbenzyloxyiminomethylcamptothecin;
- 7-[2-(2,4-Difluorphenyl)ethoxy]-iminomethylcamptothecin;
- 7-(4-t-Butylbenzyloxy)iminomethylcamptothecin;
- 7-(1-Adamantyloxy)iminomethylcamptothecin;
- 7-(1-Adamantylmethoxy)iminomethylcamptothecin;
- 7-(2-Naphthyloxy)iminomethylcamptothecin;
- 7-(9-Anthrylmethoxy)iminomethylcamptothecin;
- 7-(6-Uracyl)methoxyiminomethylcamptothecin;
- 7-(4-Pyridyl)methoxyiminomethylcamptothecin;
- 7-(2-Thienyl)methoxyiminomethylcamptothecin;
- 7-[(N-Methyl)-4-piperidinyl]methoxyiminomethylcamptothecin;
- 7-(Benzoyloxyiminomethylmethyl)camptothecin;
- 7-[(1-Hydroxyimino)-2-phenylethyl]-camptothecin.

3. Verbindungen nach Anspruch 1, ausgewählt aus der Gruppe, bestehend aus:

- 7-[N-(4-Aminobutyl)-2-aminoethoxy]-iminomethylcamptothecin;
- 7-[N-[N-(3-Amino-1-propyl)-4-amino-1-butyl]-3-aminopropyl]-iminomethylcamptothecin.

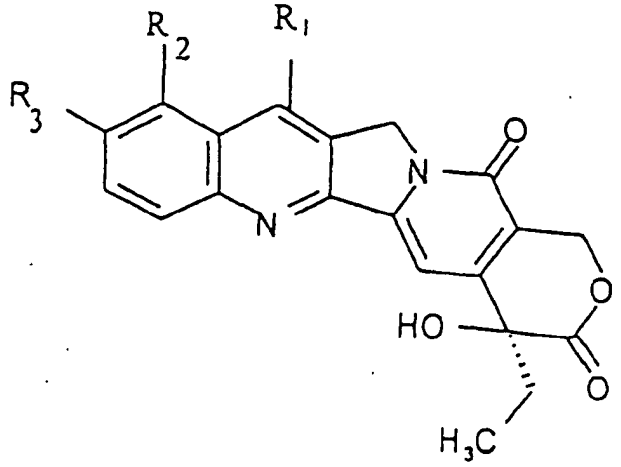
4. Verbindungen nach Anspruch 1, ausgewählt aus der Gruppe, bestehend aus:

- 7-(6-D-Galactosyloxy)iminomethylcamptothecin;
- 7-(6-D-Glucosyloxy)iminomethylcamptothecin.

5. Verbindung nach Anspruch 2, die 7-(t-Butoxy)iminomethylcamptothecin ist.

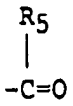
6. Verbindung nach Anspruch 2, die 7-Benzyloxyiminomethylcamptothecin ist.

7. Verfahren zur Herstellung der Verbindungen gemäß Anspruch 1, worin n 1 und R_4 wie oben definiert ist, mit Ausnahme von Aroyl und Arylsulfonyl, umfassend die Reaktion einer Verbindung der Formel (Ia)



(Ia)

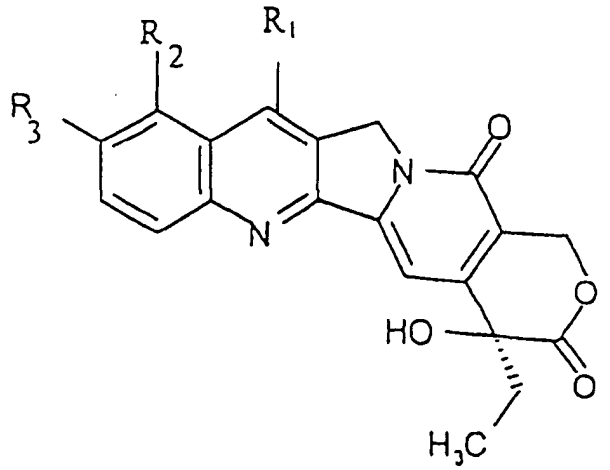
worin R_1 die Gruppe



ist und R_5 wie für die Formel (I) definiert ist, R_2 und R_3 wie in Formel (I) definiert sind, mit der Verbindung der Formel (IIa) $R_4O\text{-}NH_2$, worin R_4 wie oben definiert ist, unter Erhalt von Verbindungen der Formel (I), worin R_1 die Gruppe $-C(R_5)=N\text{-}OR_4$ ist, R_4 wie in der Formel (I) definiert ist, mit Ausnahme von Aroyl und Arylsulfonyl.

8. Verfahren nach Anspruch 7, worin das molare Verhältnis zwischen der Verbindung der Formel (Ia) und der Verbindung der Formel (IIa) zwischen 1:3 und 3:1 ist.

9. Verfahren zur Herstellung der Verbindungen gemäß Anspruch 1, worin n 1 und R_4 wie oben definiert ist, mit der Ausnahme von Aroyl und Arylsulfonyl, umfassend die Reaktion einer Verbindung der Formel (Ia)

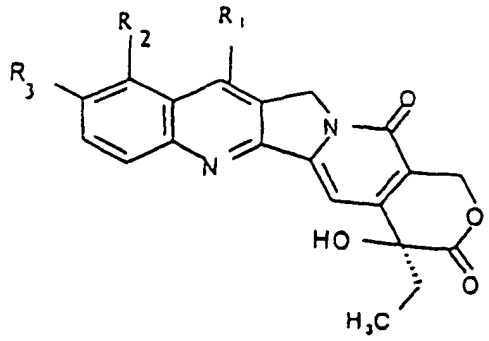


(Ia)

worin R_1 die Gruppe

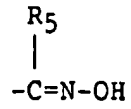
ist, und R_5 wie für die Formel (I) definiert ist, R_2 und R_3 wie in der Formel (I) definiert sind, mit einem Halogenid R_4-X, worin X ein Halogen und R_4 wie oben definiert ist, unter Erhalt von Verbindungen der Formel (I), worin R_1 die Gruppe -C(R_5)=N-OR_4 ist, R_4 wie in der Formel (I) definiert ist, mit der Ausnahme von Aroyl und Arylsulfonyl.

10. Verfahren zur Herstellung von Verbindungen gemäß Anspruch 1, worin n 1 und R_4 Aroyl oder Arylsulfonyl ist, umfassend die Reaktion einer Verbindung der Formel (Ia)



(Ia)

worin R_1 die Gruppe



ist, und R_5 wie für die Formel (I) definiert ist, R_2 und R_3 wie in der Formel (I) definiert sind, mit einem Acylchlorid R_4-COCl, worin R_4 Aroyl oder Arylsulfonyl wie oben ist, unter Erhalt von Verbindungen der Formel (I), worin R_1 die Gruppe -C(R_5)=N-OR_4 ist, R_4 Aroyl oder Arylsulfonyl ist.

11. Verbindungen nach einem der Ansprüche 1 bis 6, als Medikamente.

12. Pharmazeutische Zusammensetzung, umfassend eine therapeutisch wirksame Menge von zumindest einer Verbindung der Ansprüche 1 bis 6 in Zumischung mit pharmazeutisch akzeptablen Trägern und Exzipienten.

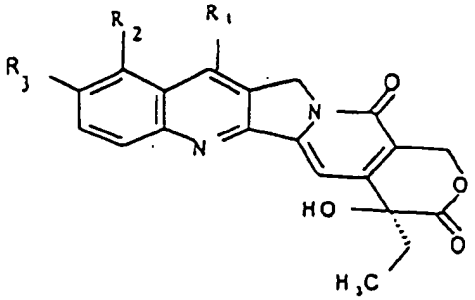
13. Pharmazeutische Zusammensetzung, umfassend eine therapeutisch wirksame Menge von zumindest einer Verbindung der Ansprüche 1 bis 6 in Zumischung mit pharmazeutisch akzeptablen Trägern und Exzipienten und wahlweise in Kombination mit anderen aktiven Bestandteilen.

14. Pharmazeutische Zusammensetzung nach Anspruch 13, worin der andere aktive Bestandteil ein Antitumormittel ist.

15. Verwendung einer Verbindung der Ansprüche 1 bis 6 zur Herstellung eines Medikamentes, das für die Behandlung von Tumoren nützlich ist.

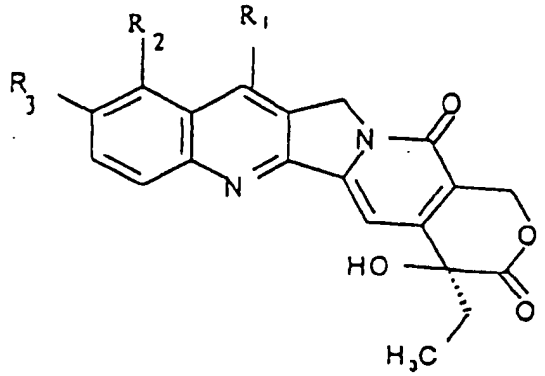
16. Verwendung nach Anspruch 15, worin der Tumor ein nicht-kleines Zelllungenkarzinom ist.

17. Verwendung von Verbindungen der Formel (Ia)



worin R_1 eine Gruppe $-C(R_5)=N-OR_4$ ist, worin R_4 Wasserstoff ist; oder eine Gruppe (C_6-C_{10})-Aroyl oder -Arylsulfonyl, wahlweise mit einer oder mehreren Gruppen substituiert, ausgewählt aus der Gruppe, bestehend aus Halogen, Hydroxy, linearem oder verzweigtem C_1-C_8-Alkyl, linearem oder verzweigtem C_1-C_8-Alkoxy, Phenyl, Cyano, Nitro, $-NR_{10}R_{11}$, worin R_{10} und R_{11}, die gleich oder verschieden sein können, Wasserstoff, lineares oder verzweigtes C_1-C_8-Alkyl sind;
R_2 und R_3, die gleich oder verschieden sind, Wasserstoff, Hydroxy, lineares oder verzweigtes C_{1-8}-Alkoxy sind; als Zwischenprodukte bei dem Verfahren gemäß Anspruch 10.

18. Verwendung von Verbindungen der Formel (Ia)

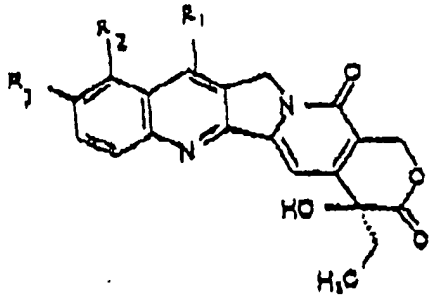


(Ia)

worin R_1 die Gruppe $-C(R_5)=O$ ist und R_5 wie für die Formel (I) definiert ist, R_2 und R_3 wie in der Formel (I) definiert sind, als Zwischenprodukte bei dem Verfahren von Anspruch 7.

Revendications

1. Composés de formule (1)



dans laquelle :

R_1 est un groupe —$C(R_5)$=N-$O_{(n)}R_4$, dans lequel R_4 est un groupe alkyle (C_1 à C_8) linéaire ou ramifié ou un groupe alkényle (C_1 à C_8) linéaire ou ramifié, ou un groupe cycloalkyle (C_3 à C_{10}), ou un groupe cycloalkyle (C_3 à C_{10})-alkyle (C_1 à C_8) linéaire ou ramifié, ou un groupe aryle (C_6 à C_{14}) ou un groupe aryle (C_6 à C_{14})-alkyle (C_1 à C_8) linéaire ou ramifié ou un groupe hétérocyclique ou hétérocyclo — alkyle (C_1 à C_8) linéaire ou ramifié, ledit groupe hétérocyclique contenant au moins un hétéroatome choisi au sein du groupe constitué par un atome d'azote, éventuellement substitué avec un groupe alkyle (C_1 à C_8) et/ou un atome d'oxygène et/ou de soufre ; lesdits groupes alkyle, alkényle, cycloalkyle, cycloalkylalkyle, aryle, aryl-alkyle, hétérocyclique ou hétérocyclo-alkyle, étant éventuellement substitués avec un ou plusieurs groupes choisis au sein du groupe constitué par ; un atome halogène, un groupe hydroxy, alkyle (C_1 à C_8), alkoxy (C_1 à C_8), phényle, cyano, nitro, -NR_6R_7, dans lequel R_6 et R_7, qui sont identiques ou différents, sont un atome d'hydrogène ou un groupe alkyle (C_1 à C_8), linéaire ou ramifié ; le groupe COOH ou un ester pharmaceutiquement acceptable de celui-ci ; ou le groupe $CONR_8R_9$, dans lequel R_8 et R_9, qui sont identiques ou différents, sont un atome d'hydrogène ou un groupe alkyle (C_1 à C_8), linéaire ou ramifié, un groupe phényle ; ou

R_4 est un groupe aroyle (C_6 à C_{10}) ou un groupe arylsulfonyle (C_6 à C_{10}), éventuellement substitué avec un ou plusieurs groupes choisis au sein du groupe constitué par : un atome halogène, un groupe hydroxy, un groupe alkyle (C_1 à C_8) linéaire ou ramifié, un groupe alkoxy (C_1 à C_8) linéaire ou ramifié, un groupe phényle, cyano, nitro, -$NR_{10}R_{11}$, dans lequel R_{10} et R_{11}, qui sont identiques ou différents, sont un atome d'hydrogène, ou un groupe alkyle (C_1 à C_8) linéaire ou ramifié ; ou

R_4 est un groupe polyaminoalkyle de formule —$(CH_2)_m$-NR_{12}-$(CH_2)_p$-NR_{13}-$(CH_2)_q$-NH_2, dans laquelle m et p sont un nombre entier compris entre 2 et 6 et q est un nombre entier de 0 à 6, inclus, et R_{12} et R_{13} sont un groupe alkyle (C_1 à C_8) linéaire ou ramifié ; ou

R_4 est un groupe glycosyle choisi parmi le 6-D-galactosyle et le 6-D-glucosyle ;

n est égal à 1 ;

R_5 est un atome d'hydrogène, un groupe alkyle (C_1 à C_8) linéaire ou ramifié, un groupe alkényle (C_1 à C_8) linéaire ou ramifié, un groupe cycloalkyle en (C_3 à C_{10}), un groupe alkyle (C_1 à C_8) linéaire ou ramifié- cycloalkyle (C_3 à C_{10}), un groupe aryle (C_6 à C_{14}), un groupe alkyle (C_1 à C_8) linéaire ou ramifié-aryle (C_6 à C_{14}) ;

R_2 et R_3, qui sont identiques ou différents, sont un atome d'hydrogène, un groupe hydroxy ou un groupe alkoxy (C_1 à C_8) linéaire ou ramifié ;

leurs N_1-oxydes, leurs isomères uniques ; en particulier les isomères syn et anti du groupe $C(R_5)$=N-$O_{(n)}$-R_4, leurs énantiomères et diastéréoisomères possibles, et les mélanges apparentés, et leurs sels pharmaceutiquement acceptables.

2. Composés selon la revendication 1, choisis au sein du groupe constitué par :

- la 7-méthoxyiminométhylcamptothécine;
- la 7-éthoxyiminométhylcamptothécine;
- la 7-isopropoxyiminométhylcamptothécine;
- la 7-(2-méthylbutoxy)iminométhylcamptothécine ;
- la 7-t-butoxyiminométhylcamptothécine ;
- la 7-(4-hydroxylbutoxy)iminométhylcamptothécine ;
- la 7-triphénylmétoxyiminométhylcamptothécine;
- la 7-carboxymétoxyiminométhylcamptothécine;
- la 7-(2-amino)étoxyiminométhylcamptothécine;
- la 7-(2-N,N-diméthylamino)étoxyiminométhylcamptothécine;
- la 7-allyloxyiminométhylcamptothécine;
- la 7-cyclohexyloxyiminométhylcamptothécine ;
- la 7-cyclohexylméthoxyiminométhylcamptothécine;
- la 7-cyclooctyloxyiminométhylcamptothécine ;
- la 7-cyclooctylméthoxyiminométhylcamptothécine ;

- la 7-benzyloxyiminométhylcamptothécine;
- la 7-[(1benzyloxyimino)-2-phényléthyl]camptothécine ;
- la 7-(1-benzyloxyimino)éthylcamptothécine ;
- la 7-(1-t-butoxyimino)éthylcamptothécine ;
- la 7-p-nitrobenzyloxyiminométhylcamptothécine ;
- la 7-p-méthylbenzyloxyiminométhylcamptothécine ;
- la 7-pentafluorobenzyloxyimmométhylcamptothécine ;
- la 7-p-phénylbenzyloxyiminométhylcamptothécine ;
- la 7-[2-(2,4-difluorophényl)éthoxy]iminométhylcamptothécine ;
- la 7-(4-t-butylbenzyloxy)iminométhylcamptothécine ;
- la 7-(1-adamantyloxy)iminométhylcamptothécine ;
- la 7-(1-adamantylméthoxy)iminométhylcamptothécine;
- la 7-(2-naphthyloxy)iminométhylcamptothécine ;
- la 7-(9-anthrylméthoxy)iminométhylcamptothécine ;
- la 7-(6-uracyl)méthoxyiminométhylcamptothécine
- la 7-(4-pyridyl)méthoxyiminométhylcamptothéclne ;
- la 7-(2-thiényl)méthoxyiminométhylcamptothécine ;
- la 7-[(N-méthyl)-4-pipéridinyl]méthoxyiminométhylcamptothécine ;
- la 7-(benzoyloxyiminométhylméthyl)camptothécine;
- la 7-[(1- hydroxyimino)-2-phényléthyl)camptothécine .

3. Composés selon la revendication 1, choisis au sein du groupe constitué par :

- la 7-[N-(4-aminobutyl)-2-aminoéthoxy]iminométhylcamptothécine;
- la 7-[N-[N-(3-amine-1-propyl)-4-amine-1-butyl]-3-aminopropyl]iminométhylcamptothécine .

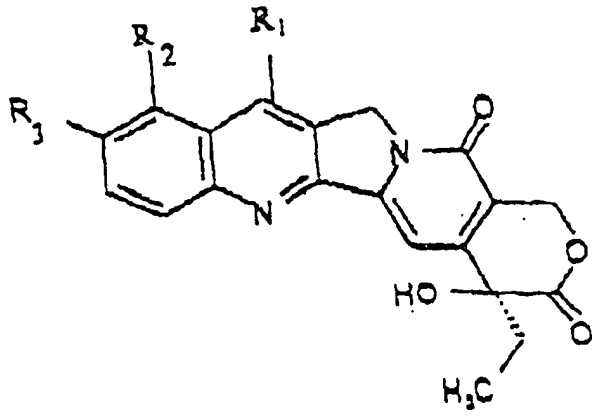
4. Composés selon la revendication 1, choisis au sein du groupe constitué par :

- la 7-(6-D-galactosyloxy)iminométhylcamptothécine ;
- la 7-(6-D-glucosyloxy)iminométhylcamptothécine.

5. Composé selon la revendication 2, qui est la 7-(t-butoxy)iminométhylcamptothécine.

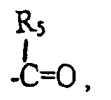
6. Composé selon la revendication 2, qui est la 7-benzyloxyiminométhylcamptothécine.

7. Procédé de préparation du composé de la revendication 1, dans lequel n est égal à un et R_4 est tel que défini ci-dessus, à l'exception des groupes aroyle et arylsulfonyle, comprenant la réaction d'un composé de formule (Ia)



(Ia)

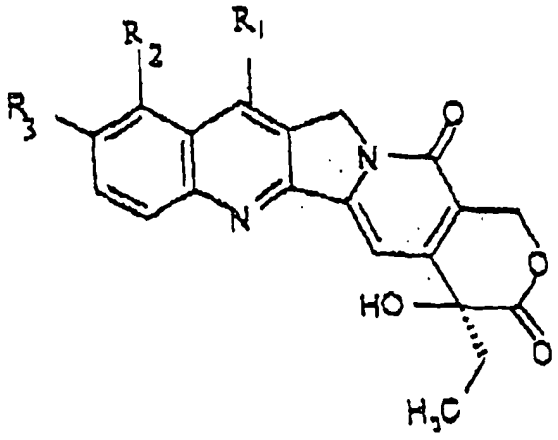
dans laquelle R_1 est le groupe



et R_5 est tel que défini dans la formule (I), R_2 et R_3 sont tels que définis dans la formule (I). Le composé de formule (Ia) est soumis à une réaction avec le composé de formule (IIa) $R_4O\text{-}NH_2$, R_4 est tel que défini ci-dessus, pour donner des composés de formule (I) dans laquelle R_1 est le groupe $—C(R_5)=N\text{-}OR_4$, R_4 est tel que défini à la formule (I) à l'exception des groupes aroyle et arylsulfonyle.

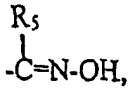
8. Procédé selon la revendication 7, dans lequel le rapport molaire entre le composé de formule (Ia) et le composé de formule (IIa) est compris entre 1 :3 et 3 :1.

9. Procédé de préparation des composés de la revendication 1, dans lequel n est égal à un et R_4 est tel que défini ci-dessus, à l'exception des groupes aroyle et arylsulfonyle, comprenant la réaction d'un composé de formule (Ia)



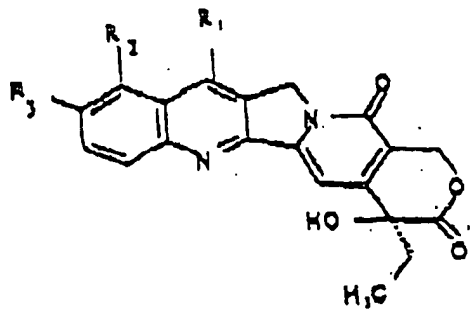
(Ia)

dans laquelle R_1 est le groupe



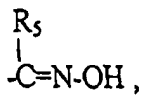
et R_5 est tel que défini dans la formule (I), R_2 et R_3 sont tels que définis dans la formule (I). Le composé de formule (Ia) est soumis à une réaction avec le composé halogénuré R_4-X, où X est un halogène pour donner des composés de formule (I) dans laquelle R_1 est le groupe $—C(R_5)=N\text{-}OR_4$, R_4 est tel que défini dans la formule (I) à l'exception des groupes aroyle et arylsulfonyle.

10. Procédé de préparation des composés de la revendication 1, dans lequel n est égal à un et R_4 est un groupe aroyle et arylsulfonyle, comprenant la réaction d'un composé de formule (Ia)



(Ia)

dans laquelle R_1 est le groupe



et R_5 est tel que défini dans la formule (I), R_2 et R_3 sont tels que définis dans la formule (I), avec un chlorure d'acyle R_4-COCl, dans lequel R_4 est un groupe aroyl ou arylsulfonyle comme ci-dessus, pour donner des composés de formule (I) dans laquelle R_1 est le groupe —$C(R_5)$=N-OR_4, R_4 étant un groupe aroyle ou arylsulfonyle.

11. Composés selon l'une des revendications de 1 à 6 en tant que médicaments.

12. Composition pharmaceutique comprenant une quantité thérapeutiquement efficace d'au moins un composé selon les revendications 1 à 6, sous forme d'un mélange avec des véhicules et des excipients pharmaceutiquement acceptables.

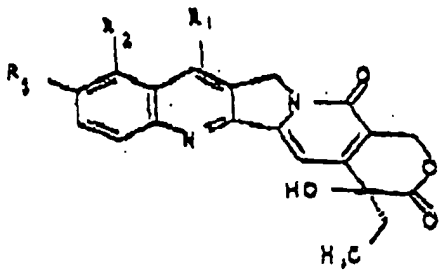
13. Composition pharmaceutique comprenant une quantité thérapeutiquement efficace d'au moins un composé selon les revendications 1 à 6, sous forme d'un mélange avec des véhicules et des excipients pharmaceutiquement acceptables, et éventuellement en association avec d'autres principes actifs.

14. Composition pharmaceutique selon la revendication 13 dans laquelle ledit autre principe actif est un agent anti-tumoral.

15. Utilisation d'un composé selon les revendications 1 à 6 dans la préparation d'un médicament utile dans le traitement des tumeurs.

16. Utilisation selon la revendication 15, dans lequel ladite tumeur est un carcinome pulmonaire non-à petite cellules.

17. Utilisation des composés de formule (Ia)



dans laquelle :

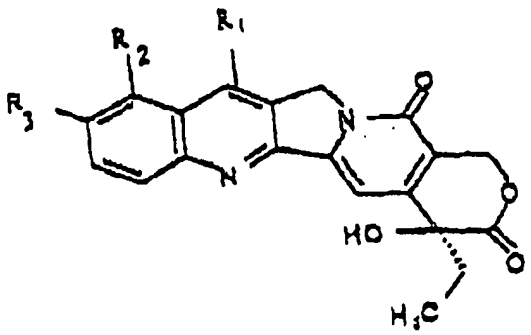
R_1 est un groupe —$C(R_5)$=N-$O_{(n)}R_4$, dans lequel R_4 est un atome d'hydrogène, ou un groupe aroyl (C_6 à C_{10})

ou arylsulfonyle, éventuellement substitué avec un ou plusieurs groupes choisis au sein du groupe constitué par : un atome halogène, un groupe hydroxy, un groupe alkyle (C_1 à C_8) linéaire ou ramifié, alkoxy (C_1 à C_8) linéaire ou ramifié, phényle, cyano, nitro, $-NR_{10}R_{11}$, dans lequel R_{10} et R_{11}, qui sont identiques ou différents, sont un atome d'hydrogène, ou un groupe alkyle (C_1 à C_8) linéaire ou ramifié;

R_2 et R_3 sont un atome d'hydrogène, un groupe hydroxy, ou un groupe alkoxy (C_1 à C_8) linéaire ou ramifié ;

en tant qu'intermédiaires dans le procédé de la revendication 10.

18. Utilisation des composés de formule (Ia)



(Ia)

dans laquelle : R_1 est le groupe —$C(R_5)$=O, et R_5 est tel que défini dans la formule (I), R_2 et R_3 sont comme défini dans la formule (I), en tant qu'intermédiaires dans le procédé de la revendication 7.